FATE AND TRANSPORT OF HERBICIDES USED IN GROWING TRANSGENIC CANOLA IN QUEBEC SOILS

by

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This thesis is dedicated to my beloved parents: My mother- Jaspal Kaur and My father- Late S. Gurjit Singh

ABSTRACT

Canola is the second most important oilseed crop after soybean. More than 90% of the canola crop grown in the world is transgenic. Before this crop is grown more extensively in Quebec, there is a need to assess environmental risks associated with genetically engineered canola, mainly Roundup Ready (glyphosate- resistant) and Liberty Link (glufosinate-resistant) varieties. We have conducted a field study to compare and contrast the fate and transport of glyphosate and glufosinate herbicides in soil (used in transgenic canola production), with trifluralin, a herbicide commonly used with non-transgenic (conventional) canola cultivars. Yield data were also collected in each test plot. Three treatments were assigned (type of transgenic canola, variety, and plant stage at which herbicide application was made), in quadruplicate, in a completely randomized block design set up at two sites: Emile A. Lods Agronomy Research Centre in the Macdonald Campus of McGill University and the Normandin site of Agriculture and Agri food Canada. Soil samples were collected from two depths, 0-0.15 m and 0.15-0.30 m, at different times (1, 7 and 20 days) after herbicide application, during the growing season. Non-significant yield differences were observed between herbicide-resistant and conventional canola grown in Quebec. Glufosinate was found to be the least persistent herbicide (half-life = 7 days), while trifluralin was found to have the longest persistence in the soil (half-life = 54 days). The overall order of persistence was Trifluralin > Glyphosate > Glufosinate. Negligible leaching was observed in case of glyphosate and glufosinate to a depth of 0.30 m. Since glyphosate was found to be highly adsorbed on to the topsoil at the Lods site, herbicide concentrations were recalculated for the top 2 mm of soil, as per published literature, and they were very high, thus heightening concerns for polluted surface runoff. Trifluralin was found at the lower depth (0.15-0.30 cm) at 7 and 20 days after herbicide application, with higher (relative to each herbicide's level in the soil one day after application) residual concentrations than herbicides used in herbicide resistant (HR) canola.

RÉSUMÉ

Le canola est au deuxième rang mondial, après le soja, comme culture oléagineuse. Plus de 90% de la récolte de canola provident de variétés transgéniques. Avant que cette culture prenne sa place au Québec il est nécessaire d'évaluer les risqué environnementaux associés au canola transgénique, en particulier les variétés Roundup Ready (tolérant au glyphosate) et Liberty Link (tolérant au glufosinate). Une étude au champ, nous a permis de comparer et mettre en contraste le devenir et le transport d'herbicides à base de glyphosate and glufosinate, utilisées dans la production de canola transgénique, dans le sol, avec la trifluraline, un herbicide servant au contrôle des mauvais herbes dans le canola non-transgénique (conventionnel). Le rendement fut évalue pour chaque parcelle. Trois traitements furent imposes (type de canola transgénique, variété, et étape de développement de la culture lors de l'application d'herbicide), avec 4 répétitions, dans un protocole complètement aléatoire et plans en blocs aléatoires complets, mis en place à deux sites: la Ferme Lods sur le campus Macdonald de l'université McGill, et au site Normandin d'Agriculture et Agroalimentaire Canada. Des échantillons de sol furent prélevés à deux profondeurs (0-0.15 m et 0.15-0.30 m), à différent moments (1, 7 et 20 jours) après l'application d'herbicide. Des différences non-significatives furent notées entre le rendement des variétés de canola résistantes aux herbicides et le canola conventionnel, cultivées au Québec. Le glufosinate se montra l'herbicide le moins persistant dans le sol (demi vie = 7 jours), tandis que la trifluraline se montra le plus persistant (demi-vie = 54 jours). Globalement, la persistance de ces herbicides suit l'ordre Trifluraline > Glyphosate > Glufosinate. Un niveau négligeable de lessivage jusqu'à une profondeur de 0.30 m fut observe pour le glyphosate et le glufosinate. Etant donné la forte liaison du glyphosate aux sols du site Lods, les concentrations recalculées pour the 2 mm en surface furent très élevées, représentant ainsi un certain risque de perte par ruissèlement. La trifluralin fut détectée dans la zone du sol la plus profonde (0.15-0.30 m) 7 et 20 jours après l'application d'herbicide application, et présenta des concentrations résiduelles plus élevés à 7 et 20 jours, sur une base relative aux niveaux présents dans le sol un jour après l'application des herbicides, que les herbicide appliqués aux variétés de canola résistantes aux herbicides.

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CHAPTER 1

INTRODUCTION

Canola (*Brassica napus* L.) is a member of family Brassicaceae (Cruciferae). Globally, it is the second most important oilseed crop after soybean [Glycine max (L.) Mill] (Pillay *et al*., 2011). Canada is the worldwide leader in canola production, producing 20% of the world's canola (information available at http://www.soyatech.com/canola_facts.htm). Canadian canola represents an annual economic market share of $13.8 billion, including $1.3 billion in Ontario and Quebec (Canola Council of Canada, 2011). Canola has been modified to be resistant to some non-selective herbicides, since these broad spectrum herbicides are the best solution to the difficult task of controlling weeds in canola. Termed herbicide-resistant (HR) or transgenic, the resultant modified canola maintains an equally consistent oil quality as its unmodified progenitor.

Herbicide-resistant canola is becoming more tempting to Canadian farmers due to its better yield, net profit and superior weed management (Fulton and Keyowski, 1999). Roundup Ready and Liberty Link are two trade names of HR crops developed through the insertion of novel gene/genes, whereas the trade name Clearfield represents crops developed by the induction of genetic mutations, namely mutagenesis (Brimner *et al*., 2005). These respective genetic manipulations have introduced resistant gene(s) conferring resistance to glyphosate [*N*-(phosphonomethyl)glycin], glufosinate [ammonium (2*RS*)-2-amino-4-(methylphosphinato) butyric acid] and imizathapyr [5-ethyl-2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid], herbicides abundantly used in canola production in Canada (Beckie *et al*., 2006). Amongst HR crops, glyphosate-resistant (GR) cultivars are widely planted; for example, 62% of canola planted in the US in 2005 was GR (Sankula, 2005). In 2010, the total area under (GM and non GM) canola was 6.5×10^6 ha in Canada, and rose to which became 7.5×10^6 ha in

2011 (Statistics Canada, 2011). Continuously increasing demand for canola may be attributable to the Canadian Government's decision to use 2% biodiesel as a renewable fuel in the country by July, 2011 (Canola Council of Canada, 2011). The recent establishment of the Government-assisted canola seed crushing facility in Becancour, Quebec by TRT-ETGO, has proven to be beneficial in opening new avenues for enhancing canola cultivation in eastern Canada (Business Wire, 2008).

Consequently, increased canola acreage can put extra herbicide load into the different compartments of the environment and ultimately cause environmental pollution. Of the total pesticides (4.17 Gg yr^{-1}, as active ingredient) presently sold in Quebec, 59.6% are herbicides, 16.9% fungicides and 12.5% insecticides (Gorse and Rivard, 2011). Among the total Canola acreage in Canada, 99% is under herbicide resistant cultivars (Martino-Catt *et al*., 2012). In spite of the introduction of number of GM cultivars, limited information is available on the indirect impacts of HR crops on soil contamination through heightened application of specific pesticides. With the increasing diversity of chemicals and cultivars, new problems may arise, which are sometimes difficult to predict. In case of GM crops, the same resistant gene is used in several crops; as a result the same herbicide is extensively used against weeds. Such an extensive use of the same herbicide can lead to develop resistance in weeds (Ministry of Agricuture, Food and Rural Affaires, Ontario, 2011) and also a decline in pricing, leading farmers to apply overdoses of herbicide where some weeds become more troublesome (Medsen and Streibig, 2003). This can pose a threat to surface water bodies and ground water. Glyphosate and glufosinate have been extensively studied in laboratory and forest soils, but there is very limited information available in field soils. Hence special attention must be given to their possible persistence and transport in

Quebec soils at the field level, compared to the herbicides which they replace (e.g. trifluralin [α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-p-toluidine]).

The primary concern in exploring the fate and transport of these chemicals is in developing the information needed to assure that herbicides used in weed control in transgenic canola do not create problems in the soil environment. However, it has been argued that increased use of herbicide-resistant crops can pose potential environmental effects from the herbicides used in HR crops (Haney et al., 2000), and may increase the transfer of herbicides to water bodies either by surface runoff or by vertical movement into ground water (Mamy et al., 2005). Torstensson *et al.* (1989) studied the influence of climatic conditions on the persistence of glyphosate herbicides in the southern and northern parts of Sweden, and reported significant differences. Although plenty of literature has been published concerning the fate and transport of herbicides used in HR crops in the controlled environment of laboratories, little information is available about the fate of these herbicides in cultivated soils.

The behaviour and fate of these herbicides should be evaluated in the context of Quebec soils and agriculture, before increasing the area under Canola. This research project is conducted to evaluation the environmental fate and transport of herbicides used in herbicide-resistant canola in Quebec.

1.1 Based on the above discussion; this research project is conducted to fulfill the following objectives:

1. Relative comparison of herbicide-resistant canola and conventional canola cultivars based on the yield.

2. Environmental fate of herbicides used in herbicide resistant canola in southwestern Quebec soils compared with the trifluralin herbicides used in conventional canola.

1.2 Thesis Organization

This thesis has five chapters. Chapter 1 deals with introduction to research topic and this is followed by literature review in Chapter 2. All the materials and methods used to achieve the objectives of this study are discussed in Chapter 3. In Chapter 4, details of the results obtained in this study are presented and discussed. This is followed by a summary and conclusions chapter. References are provided in chapter 6. Last but not least, Appendices contain dissipation graphs and weather data.

1.3 SCOPE

This research project mainly deals with the study of persistence and movement of glyphosate and glufosinate herbicides used in growing transgenic canola in Quebec. The results were obtained for a silt loam and a sandy loam soil. Therefore, care should be used in extrapolating these results to other soil types.

CHAPTER 2

REVIEW OF LITERATURE

Given their contribution to enhancing agricultural production and food quality, pesticides play an important role in current conventional agricultural practices. Agriculture is the most prominent market sector in terms of its demand for pesticides. Pesticide is a broad term used to represent three main types of chemicals employed in crop protection, viz. insecticides, fungicides and herbicides, of which the latter ranks first in importance (Zhang *et al*., 2011). North America is considered an industry leader, marketing 40% of all total herbicides worldwide (Vasilescu and Medvedovici, 2005). Herbicides are mainly used to check the growth of problematic weeds during the production seasons of soybeans and other oilseeds, corn (*Zea mays* L.), cereals, rice (*Oryza sativa* L.), fruits and nuts, and cotton (*Gossypium hirsutum* L.). Moreover, the use of some non-selective herbicides has increased dramatically since the introduction of HR crops resistant to these specific herbicides (e360 digest, 2009). Hence, intensive usage of herbicides might cause environmental pollution, since a fraction of the applied herbicide may always move away from the site of their target application: (i) to the soil where it may damage sensitive crops, (ii) to water bodies, where it may lead to deterioration of water quality, or (iii) into the atmosphere in gaseous form. To understand herbicide behaviour into the environment, a thorough knowledge of their fate and transport should be reviewed. In this chapter, general processes involved in the environmental fate of pesticides are briefly discussed and then information about each herbicide used in this study is provided with regards to its fate in the environment.

2.1 Persistence of pesticides/herbicides in soils

The time for which the herbicide remains active in the soil is known as 'soil persistence'. Differences in herbicide persistence depend mainly upon three factors: soil, climate and herbicide properties. Soil factors determining herbicide persistence include soil composition (physical factor), soil chemistry (chemical factor) and soil microbial activity (biological factor). Soil composition determines the phytotoxicity, behaviour and persistence of herbicide through three main interacting processes: adsorption, leaching and volatilization. Highly adsorbed herbicides are resistant to leaching and volatilization (even when the herbicide is volatile in nature). Moreover, soil organic matter (SOM) content also plays an important role in adsorption phenomena. However, in general, the more strongly the herbicide binds to the soil, the greater will be its persistence and its potential to injure successive crops, sensitive to that herbicide. Soil water content also affects the process of pesticide sorption to soil particles, and thus of a chemical molecule's ability to move through the soil profile. Water molecules often compete with pesticide molecules for the sorption sites on soil and organic matter. Consequently greater sorption can be expected in dry soils than in moist soils (Whitford, 2001).

Furthermore, soil's chemical properties also contribute significantly to 'soil persistence'. A laboratory study of Canadian prairie soils revealed that soil pH and organic matter content had significant effects on the dissipation of sulfentrazone [2',4'-dichloro-5'-(4-difluoromethyl-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)methanesulfonanilide] herbicides. Lesser phytotoxicity was observed with decreasing pH and vice-versa (Szmigielski *et al*., 2012). Similarly, another laboratory study investigated the effect of pH and temperature on the degradation of thiram [amethylthiuram disulfide]. At a soil pH of 5.1, the half-life of thiram was 7.7 days and decreased significantly to 4.6 days at pH of 8.1 (Gupta *et al*., 2012).

The soil microbial population takes an important part in the decomposition/breakdown of herbicides. The extent of decomposition depends on their type, number and other environmental factors affecting their growth. Reddy and Reddy (2012), while studying the microbial degradation of pyridines (heterocyclic compounds), concluded that *Arthrobacter*, *Nocardia*, *Micrococcus*, and *Bacillus sp*. were beneficial in the bioremediation of these compounds. Thus a specific bacterial species can efficiently degrade a specific chemical. Factors that affects the degradation process of herbicides are environmental and non-environmental. Major environmental factors include temperature, pH, oxygen availability and moisture content of the soil, whereas non-environmental factors include herbicide solubility, lipophilicity, molecular size and volatility (Sinha *et al*., 2012).

2.2 Herbicide dissipation

Dissipation refers to the breakdown of a herbicide to the extent that it no longer possesses herbicidal activity and by that time the original molecule has largely released into the environment as carbon dioxide. The main processes involved in the dissipation of a herbicide include microbial activity, photolysis and hydrolysis. Weakly adsorbed herbicides are frequently bioavailable and are thus available to the microbial population for degradation (Basham and Lavy, 1987). Generally, strong sorption can be expected from dry (*vs.* moist) soil, so soil moisture content has a strong influence in determining dissipation (Graebing *et al*., 2003). Under conditions of low soil moisture content, herbicide transport is minimal, leading to minimal interaction between microorganisms and the herbicide, which, in turn, results in less degradation and longer persistence (Shelton and Parkin, 1991). In another study, Saratovskikh *et al*. (2007) examined the photolytic degradation of 3, 6-dichloropicolinic acid (Lontrel herbicide). While this herbicide is not substantially degraded by microorganisms, photolysis of this herbicide was found to result in the formation of metabolites, as well as lingering un-degraded parent herbicide (Saratovskikh *et*

al., 2007). Similarly, both microbial and photolytic pathways of degradation were studied for acetochlor herbicide and it was found that microbial pathway results in less phytotoxic products while one of the photolytic degradation products showed equally comparable phytotoxicity as acetochlor (Jablonkai, 2000). Generally, microbial degradation is a relatively more efficient mode of degradation compared with photolysis for most of the herbicides (Tomco and Tjeerdema, 2012). Photolytic degradation of herbicides has considerable importance in herbicidal dissipation, particularly in regions with high UV radiation intensity (Ramezani *et al*., 2008). Pesticides can also be degraded by the process of hydrolysis, by which water reacts with the molecule, resulting in the breakdown of pesticides into smaller molecules. However, rate of hydrolysis is greater at the soil surface as the water is warmer than at greater soil depths.

2.3 Environmental fate of glyphosate

Glyphosate is a broad-spectrum non-selective herbicide used to control weeds, mainly annual broad leaf weeds, grasses and woody plants. After being absorbed by the plant, it moves through the plant from the point of contact into the root system. It is highly soluble in water but poorly soluble in organic solvents (Sanchis *et al*., 2012). A maximum drinking water contaminant level (MCL) of 700µg L^{-1} has been assigned by the USEPA. Long term exposure above the MCL can effects kidneys and reproductive systems in human beings (EPA, 2012). In Canada, the interim maximum allowable concentration (IMAC) in drinking water is 280µg L^{-1} (Health Canada., 1995). The value set by Canadian Council of Ministers of the Environment for the protection of aquatic life is 65µg L^{-1} (CCME, 2012). The structural formula of glyphosate and its physical and chemical properties are shown in Fig. 2.1 and Table 2.1, respectively.

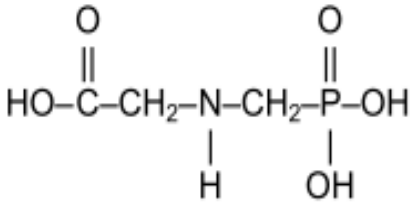

Figure 2.1 Structural formula of glyphosate

(Ref: http://www.inchem.org/documents/pds/pds/pest91_e.htm)

Table 2.1: Physical and chemical properties of glyphosate (University of Hertfordshire,2012)

Chemical Name	***N*-(phosphonomethyl)-glycine**
Molecular Formula	$C_3H_7NO_5P$
CAS No.	1071-83-6
Molecular weight	169.07 g
Melting point	189.5ºC
Appearance	Colorless crystal at room temperature
Solubility	In water, 10.5 g L^{-1} at 20ºC Almost insoluble in organic solvents
Vapor pressure	0.0131 mPa at 25ºC
Relative density	1.71g mL^{-1}
Henry's law constant	6.60×10^{-19} at 20ºC
Log K_{ow} at pH 7@20ºC	Less than -3.2
Average soil half-life (field)	12 days
Mobility Potential	Low

Mineralization is the foremost dissipation pathway for glyphosate. Microbial degradation plays a major role in the mineralization of glyphosate, since the C-P bond is resistant to physicochemical degradation (Forlani *et al*., 1999; Strange-Hensen *et al*., 2004; Sviridov *et al*., 2012), whereas chemical and photo-degradation have little significant effect on glyphosate degradation. Although the rate of mineralization depends on many biotic and abiotic factors, population growth and the presence of specific microbial species or strains are essential to the successful degradation of the compound (Sorensen *et al*., 2006). Some studies have indicated that soil's strong sorption capacity for glyphosate, results in its low mineralization/degradation (Moshier and Penner, 1978; Strange-Hensen *et al*., 2004; Gimsing *et al*., 2004).

Glyphosate is known to be degraded by two pathways, and though both pathways require a common enzyme (C-P lyase) for the cleavage of the C-P bond, the difference is in the intermediate products formed after the cleavage. Both CO_2 and NH_3 are among the final products. One pathway forms sarcosine [2-(Methylamino)acetic acid] and glycine whereas the other forms AMPA [2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid] and glyoxylate (Borggaard and Gimsing, 2008). AMPA has been often found in soils, which could be due to the high sorptive property of AMPA through the phosphonate group (Roy *et al*., 1989) as shown in Figure 2.2.

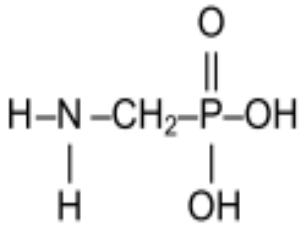

Figure 2.2 Structural formula of AMPA

(Ref: http://www.for.gov.bc.ca/hfp/publications/00015/6-Dost-Glyphosate.pdf)

The effect of soil phosphorous on the degradation/mineralization of glyphosate has been studied extensively since the glyphosate molecule resembles that of inorganic phosphate (Hance, 1976; Sprankle, 1975), so one can expect the same affinity of sorption of glyphosate with soil minerals, as occurs with phosphate (De Jonge *et al*., 2001; Gimsing *et al*., 2004; Borggaard *et al*., 2005; Gimsing *et al*., 2007; Laitinen, 2009). In general, as soil sorption increases, glyphosate degradation decreases. However, several investigations have reported discrepancies with the above generalization. This is explained by the existence of two types of sorption sites, common and specific. Common sites are available to both glyphosate and phosphate, but the phosphate is always preferred by common sites (Hill, 2001). Hence, addition of phosphorous to soil decreases the adsorption of glyphosate (Wang *et al*., 2005). On the other hand, specific sites are reserved for specific individual molecules, as the name indicates (Borggaard, 2011). Most current research findings suggest a positive correlation between phosphate and glyphosate mineralization.

Kim *et al*. (2011) studied the effect of Copper (Cu^{2+}) and Zinc (Zn) in two soils (sandy clay loam and silty clay loam) on the mineralization of glyphosate. They found that high Cu inhibited mineralization in both soils, whereas high Zn had almost no effect in either. On the other hand, Moshier and Penner (1978) observed a reduction in mineralization in the presence of iron (Fe) [Fe^{2+} or Fe^{3+}] and aluminum (Al), manganese (Mn^{2+}) stimulated glyphosate degradation whereas calcium (Ca) and sodium (Na) showed no effect on the degradation process. Degradation of glyphosate is also temperature-dependent, as lower temperatures decreases the rate of degradation (Heinonen, 1989), probably due to low microbial occurrence or activity.

Soil moisture content can also be considered an important factor contributing to chemical degradation in soil, and temperature can playing a decisive role in the determination of soil moisture status. Schroll *et al*. (2006) reported a study in which they appraised the effect of soil

moisture content on the degradation of three pesticides: isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea], benazolin-ethyl [ethyl 4-chloro-2,3-dihydro-2-oxo-1,3-benzothiazol-3-ylacetate] and glyphosate). They concluded that while a lower (more negative) water potential (ψ) reduced mineralization, poor pesticide degradation also occurred in soils at field capacity (ψ_{fc}). Optimum degradation was observed to occur in a specific range of soil moisture contents ($0.015 < \psi < -20$ MPa).

The risk of ground water and surface water contamination of glyphosate is determined by its potential of leaching and movement through the soil. The most commonly-known paths of transportation of glyphosate are in solution in water and attached to soil particles (also called particle-facilitated transportation). Although specific biotic and abiotic factors can contribute to a greater mobility of glyphosate; however, most of the literature supports the fact that competition of glyphosate with phosphate for the same sorption sites and the amount of rainfall after its application are the primary factors leading to the mobility of the herbicide (Vereecken, 2005).

From their study on glyphosate transport in undisturbed columns bearing sandy loam and sandy soil top-soils, De Jong *et al*. (2000) concluded that phosphorous concentration and pH of the soil solution did not significantly influence glyphosate leaching, whereas preferential flow and colloids facilitated transport were the major mechanism determining glyphosate leaching.

In another study, Zhou *et al*. (2010) also conducted an experiment with sandy soil columns to determine the leaching potential of atrazine and glyphosate herbicides. After 160 hours, almost 100% atrazine and but only 16% of glyphosate were recovered throughout the soil column. They concluded that atrazine presented a higher risk for contaminating water bodies.

Studying the role of soil organic matter (SOM) in the sorption of glyphosate, Albers *et al*. (2009) found that major components influencing the sorption of glyphosate include SOM, Al/Fe oxides and clay mineral content, while low pH had a less-pronounced influence. In the six soils they studied, maximum adsorption occurred in a soil with high organic matter, low pH and a high Fe- and Al-oxide content. However, they failed to explain the interaction of all these factors on the ultimate sorption of glyphosate to the soil. However, the weak bonding, which occurs between glyphosate and purified humic substances, clearly indicated the inconsequential role of soil organic matter in the sorption of glyphosate. Given the inverse relationship between pH and glyphosate adsorption, pH could have considerable impact on the mobility of glyphosate (Zhao *et al*., 2009).

Gjettermann *et al*. (2011) studied desorption of glyphosate and its mobility in soil and through particle-facilitated transport resulting from splash erosion. They observed similar desorption processes from different types of particles, and concluded that desorption kinetics could be an important parameter in routing glyphosate between dissolved and particle-facilitated transport.

Similarly, Bergström *et al*. (2011) conducted a laboratory and lysimeter experiment to evaluate the risk of glyphosate leaching in clay and sandy soils. They reported that glyphosate residues mainly stayed in the top layer of the soil. Less leaching was observed in the sandy soil than in the clay soil, and no leaching of AMPA, a major metabolite of glyphosate, occurred in the sandy soil. In contrast, glyphosate and AMPA leaching, as well as persistence, were monitored in boreal sandy soils (Laitinen *et al*., 2009). They raised the concern that significant glyphosate leaching may occur in P-rich and unploughed soils after herbicide application, posing an

environmental pollution risk due to surface runoff. A significant correlation between glyphosate load and total phosphorus load was observed in surface runoff.

Environmental concerns regarding glyphosate are lesser than those associated with most other herbicides used in growing field crops because of its high sorption to soil particles, low potential for leaching, shorter half-life, as well as its more efficient control of weeds (fewer applications needed) (Cerdeira, 2006). However, one cannot neglect the fact that climatic conditions, soil properties and the quantity and quality of soil microbial communities can influence the potential impacts of glyphosate on the environment (Ermakova *et al*., 2010; Eberbach, 1998).

2.4 Environmental fate of Glufosinate

Glufosinate is a non-selective, contact herbicide used to control both annual and perennial broadleaf along with grassy weeds (Corbett *et al*., 2004). Glufosinate acts through its inhibition of glutamine synthetase, responsible for the nitrogen metabolism in plants (Shin *et al*., 2011). This herbicide is also used as a crop desiccant. Glufosinate ammonium became much more popular in Canada after the introduction of Liberty Link crops by Bayer Crop Science in 1995 (Stringam *et al*., 2003). Liberty Link canola is resistant to glufosinate ammonium herbicide, the resistance being conferred by two bacterial genes, *bar* (bialaphos resistance) or *pat* (phosphinothricin acetyltransferase), derived from the soil bacteria *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*, respectively. Unlike most other commonly used herbicides, the USEPA has not fixed an MCL for glufosinate ammonium (Shipitalo *et al*., 2008).

As the chemical structure of glufosinate (Fig. 2.3) resembles that of glyphosate, so do its chemical properties. Like glyphosate, glufosinate is soluble in water and insoluble in non-polar

organic solvents, however; unlike glyphosate's structure, the P-atom is surrounded by two, rather than one, carbon atoms. Compared to glyphosate, this property makes glufosinate less sorptive to soil particles. Furthermore, the positive charge on the molecule leads to a weak affinity towards coarser soils due to limited cation exchange sites on these particles (Laitinen *et al*., 2008). The physical and chemical properties of glufosinate are given in Table 2.2.

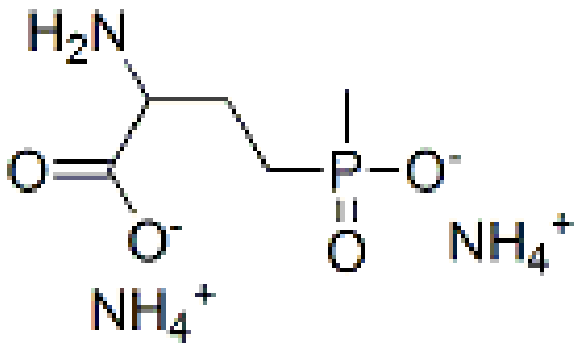

Figure. 2.3 Structural formula of glufosinate ammonium

(Ref: http://www.chemicalbook.com/ChemicalProductProperty_EN_CB2697882.htm)

Compared with glyphosate, the environmental fate of glufosinate ammonium is poorly understood. Nonetheless, studies have raised few environmental concerns regarding its fate in soil and water (Behrendt *et al*., 1990; Tebbe and Reber, 1991; Allen-King *et al*., 1995; Faber *et al*., 1997; Autio *et al*., 2004; Accinelli *et al*., 2004; Laitinen *et al*., 2006, 2008; Zablotowicz *et al*., 2008; Dinehart *et al*., 2009; Gregoire *et al*., 2010).

Table 2.2: Physical and chemical properties of glufosinate ammonium (University of Hertfordshire, 2012)

Chemical Name	**Ammonium-DL-homoalanin-4-yl(methyl)phosphinate**
Molecular Formula	$C_5H_{15}N_2O_4P$
CAS No.	77182-82-2
Molecular weight	198.2 g
Melting point	216.5 ºC
Appearance	White crystalline powder
Solubility	0.50 kg L^{-1} at 20ºC
Vapor pressure	3.10×10^{-2} at 25ºC
Relative density	1.32 g mL^{-1}
Henry's law constant	$4.48x10^{-9}$ at 25ºC
Log K_{ow}	-4.01 at pH 7
Average soil half life	Days to few weeks (Carpenter and Boutin, 2010)
Mobility Potential	Medium to high

Like glyphosate, glufosinate is mainly degraded by soil microbes. Moist soils with high organic matter tend to exhibit a greater rate of glufosinate mineralization due to greater microbial growth (Behrendt *et al*., 1990). As mentioned in various studies, glufosinate ammonium degrades into 3-methylphosphinylpropionic acid (MPPA-3), which further undergoes degradation into 2-

methylphosphinylacetic acid (MPPAA-2). Faber *et al*. (1997), studying the degradation and persistence of glufosinate ammonium and its metabolites in the forests of northern Ontario, found 95% of the parent compound to be present in the 0-0.10 m soil profile, which corresponded with the layer bearing the greatest organic matter. The metabolite MPPA-3 was found even on the same day as glufosinate application occurred, with less than 5% being found below 0.10 m depth. In their study, the half-life of glufosinate was been found to be 4.3 days. However, some studies reported its half-life to range from 1 to 10 days in sandy loam soils (Gallina and Stephenson, 1992; Behrendt *et al*., 1990), and up to 32 days in clay loam soils (Devos *et al*., 2008).

Siimes *et al*. (2006) compared the behaviour of glyphosate, glufosinate and ethofumesate [(*RS*)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate] under bare field conditions, and reported that all three herbicides remained in the topsoil. In the same experiment, herbicide loads in surface runoff and subsurface drainage were determined during the first 302 days after application. Maximum runoff losses were found after the snowmelt and soil-thaw. Reported losses over the 302 days after herbicide application were 1%, 0.2% and 0.1% for ethofumisate, glufosinate and glyphosate, respectively. In this study glyphosate and its metabolite AMPA were not detected and quantified from the soil samples due to interference with other peaks appearing at the same retention time. A similar study, regarding glyphosate and glufosinate ammonium runoff losses from a corn field in Italy, revealed that there was a low potential of water contamination from these herbicides, given their short half-life and strong sorption to soils (Screpanti *et al*., 2005). The highest concentrations (in drainage water) in this study were 16 and 24 μg L^{-1} for glyphosate and glufosinate, respectively, observed after 1 day of herbicide application.

Autio *et al*. (2004) studied the adsorption of three herbicides used in conventional sugar beet (*Beta vulgaris* L.) cultivation and two herbicides used in herbicide resistant sugar beet

cultivation (viz. glyphosate and glufosinate) in Finnish soils. They found the adsorption of glufosinate ammonium (based on K_f or K_d values) had no correlation with SOM; however, it did correlate with the soil's clay content. Other factors like pH, mineral composition — particularly phosphorous content — of the soil were found to have a significant effect on the adsorption and movement of glyphosate. The adsorption coefficient derived from this study indicated that glufosinate ammonium exhibited high mobility in most soil types.

Ahmad and Malloch (1995) found, while conducting an experiment to assess the impact of phosphinothricin (a.k.a. glufosinate) at concentrations ranging from 0 to 50 mM on soil microorganisms, varying levels of tolerance amongst different microorganisms. However, the presence of 1 mM phosphinothricin in agricultural soils can decrease the fungal population by 20% and that of bacteria by 40%. The authors were unsure of the possible outcomes of alterations in the soil microbial communities, but raised the concern of such compounds being responsible for alterations in soil ecosystems. A similar study by another research group identified three glufosinate ammonium tolerant bacterial species: *Burkholderia sacchari*, *Serratia marcescens* and *Pseudomonas psychrotolerans.* They suggested that long-term exposure to glufosinate could generate tolerant bacterial strains with significant degradation efficiency (Hsiao *et al*., 2007).

Glufosinate ammonium is considered to be mobile in most soils, except volcanic ash. The persistence of glufosinate depends on many factors such as climate and many other environmental and soil-related characteristics. However the USEPA, drawing on many studies reported its half-life ranging from 12 to 70 days and designated glufosinate as a persistent herbicide. Its major metabolite, 3-methylphosphinicopropionic acid, was found to be more persistent and mobile than its parent compound (Cox, 1996).

2.5 Environmental fate of trifluralin

Trifluralin (α, α, α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) is a pre-emergence herbicide (*i.e*., herbicide applied prior to weed seedling emergence), selective herbicide, widely used to control broadleaf weeds, particularly in canola (Mamy *et al*., 2008), sunflower (Helianthus annuus L.), vegetable crops and many annual grasses. The herbicide is incorporated into the soil, thus its main target is the plant-root system. The phytotoxicity of trifluralin occurs by its inhibition of the root growth, through disruption of root cell mitotic activity by hindering microtubule development during mitosis (Hess and Bayer, 1977). Investigations of trifluralin's persistence and dissipation in different soils have shown that soil moisture, temperature, texture, pH and depth of incorporation influence its degradation (Jolley and Johnstone, 1994; Savage, 1973; Miller *et al*., 1975). Since trifluralin has been declared a persistent bioaccumulative toxin (PBT) in the Persistence and Bioaccumulation Regulations of CEPA (1999), its environmental fate has been studied by many environmental researchers. Canadian drinking water quality guidelines for the protection of agricultural water of 45 µg L^{-1} have been suggested for trifluralin (CCME, 1999). The structural formula of trifluralin is presented in Fig. 2.4 and its physical and chemical properties are shown in Table 2.3.

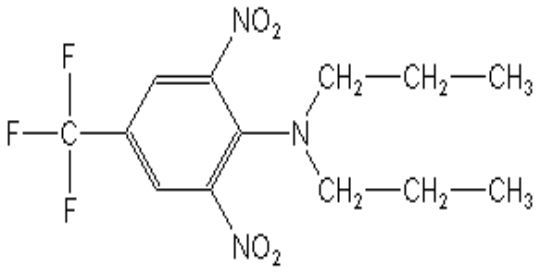

Fig.2.4 Structural formula of trifluralin

(Ref: http://www.alanwood.net/pesticides/trifluralin.html)

Table 2.3: Physical and chemical properties of trifluralin (University of Hertfordshire, 2012)

Chemical Name	**2,6-dinitro-N,N-dipropyl-4-trifluoromethylaniline**
Molecular Formula	$C_{13}H_{16}F_3N_3O_4$
CAS No.	1582-09-8
Molecular weight	335.28 g
Melting point	47.2 ºC
Appearance	Bright orange crystalline solid
Solubility	Slightly soluble in water (0.221 mg L^{-1})
Vapor pressure	9.5 mPa at 25ºC
Relative density	1.36 g mL^{-1}
Henry's law constant	4×10^{-2} at 20 ºC
Log K_{ow}	5.27 at 20 ºC
Average soil half life	53.3-56.8 days (Triantafyllidis *et al*., 2010)
Mobility Potential	Low

Persistence and degradation of herbicides play a vital role in risk assessments studies (Mamy et al., 2005), as it is undesirable for these herbicides to remain in the environment sufficiently long as to affect subsequent sensitive crops. Chemicals with a long persistence can pose undesirable risks to the surrounding environment, including water bodies. The major dissipation process for trifluralin is volatilization (Triantafyllidis *et al*., 2010) and photo-degradation (Savage, 1973; Johnstone *et al*., 1998). However, other dissipation pathways like

bacterial degradation and chemical degradation are known to exist to some extent (Parka and Tepe, 1969). A field study showed 98% volatilization losses from trifluralin applied to the soil surface in the first 6 days before the herbicide was incorporated into the soil. After the incorporation of the herbicide, very low volatilization was reported and the amount of trifluralin in the soil continued to decrease after 101 days from its application suggesting that other biotic and abiotic factors were contributing towards its disappearance, particularly soil moisture content (Bedos *et al*., 2006). Dimou *et al*. (2004), investigating the photo-degradation of trifluralin in soil and the effect of SOM on this process, found that SOM content has a positive effect on the degradation kinetics of trifluralin in soil, though its role in enhancing photodegradation was not entirely clear.

While observing microbial degradation of trifluralin, Bellinaso *et al*. (2003) isolated soil bacteria, resistant to trifluralin. Out of the nine bacteria isolated, four identified (*Klebsiella sp*., *Herbaspirillum sp*., and two strains of *Bacillus sp*.) and one unidentified bacteria were found to be competent in trifluralin degradation. The study also showed differences in degradation efficiency, according to the composition of the culture medium.

In most pesticide degradation processes the rate of microbial degradation is positively influenced by temperature (Lu *et al*., 2006). A similar trend was observed while studying the effect of solarization and biosolarization on the dissipation of trifluralin herbicide in soil. As a result of elevated soil temperatures arising from trapping of radiant energy from the sun, an increase in dissipation rate was observed using biosolarization disinfection technique (Fenoll *et al*., 2010).

2.6 Extraction of herbicides

Extraction of herbicide from a complex media such as soil is a critical step prior to the injection of a sample into the column. A number of techniques for the extraction of herbicides from soil have been extensively discussed by the scientific community (Andreu and Picó, 2004; Liang and Hay, 2011; Lourencetti *et al*., 2008; Martínez *et al*., 2009). The chemical properties (*e.g*., vapor pressure, solubility, hydrophobicity and acid dissociation) of the herbicide(s) play an important role in understanding the basic theory behind the appropriate extraction. The importance of these properties in the transport of chemicals in soil, air, water and also immiscible phases of analytical extraction, have been discussed in detail by Wells (2003).

Liquid-liquid extraction and liquid-solid extraction are the most frequently used techniques for the extraction of organic contaminants; however, the former is limited to water-immiscible samples. In the current study, we are dealing with the soil samples, so liquid-solid extraction will be discussed in detail.

Liquid-solid extraction refers to the process of removing or extracting solutes from a solid medium by means of solvent. In solid-liquid extraction, Soxhlet extraction (i.e., USEPA method 3540) is the commonly used method for removing pesticides in soil (Marchese *et al*., 2001). This method involves use of strong organic solvents, which creates difficulty in solubilizing humic matter (Andreu and Picó, 2004). In a recent study, accelerated solvent extraction (ASE), ultrasonic extraction (UE) and agitation by magnetic stirrer were compared to evaluate the efficiencies of glyphosate, AMPA and glufosinate extraction. Of these methods, agitation with water as an extraction solvent showed the best efficiency as fewer impurities were observed, unlike procedures using strong-bases (high-molarity) for extraction (Druart *et al*., 2011).

2.7 Summary

Canola cultivation in Quebec needs to expand due to the introduction of a crushing facility in the province which requires high local canola production. Most canola cultivars grown these days are herbicide-resistant. Glyphosate and glufosinate are the herbicides, actively used in roundup ready and liberty link canola cultivars, to control troublesome weeds. In this field study, the behaviour and fate of these herbicides has been evaluated in the context of Quebec soils and climate. While reviewing the literature, it was found that very limited research has been conducted to study their fate and transport at field-scale although extensive research has been done at the laboratory and lysimeter scale.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Setup

Field trials were conducted at two sites in the summer of 2011: The Soils and Crops Research and Development Centre at Normandin, QC (Normandin site) and the Emile A. Lods Agronomy Research Centre on the Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue, QC (Lods site). Two Roundup Ready (45H28, 45H29) and two Liberty Link (5030, 5040) canola varieties, along with a conventional canola variety, Avalanche, as a check (see figure 3.1), were planted in a randomized complete block design (RCBD).

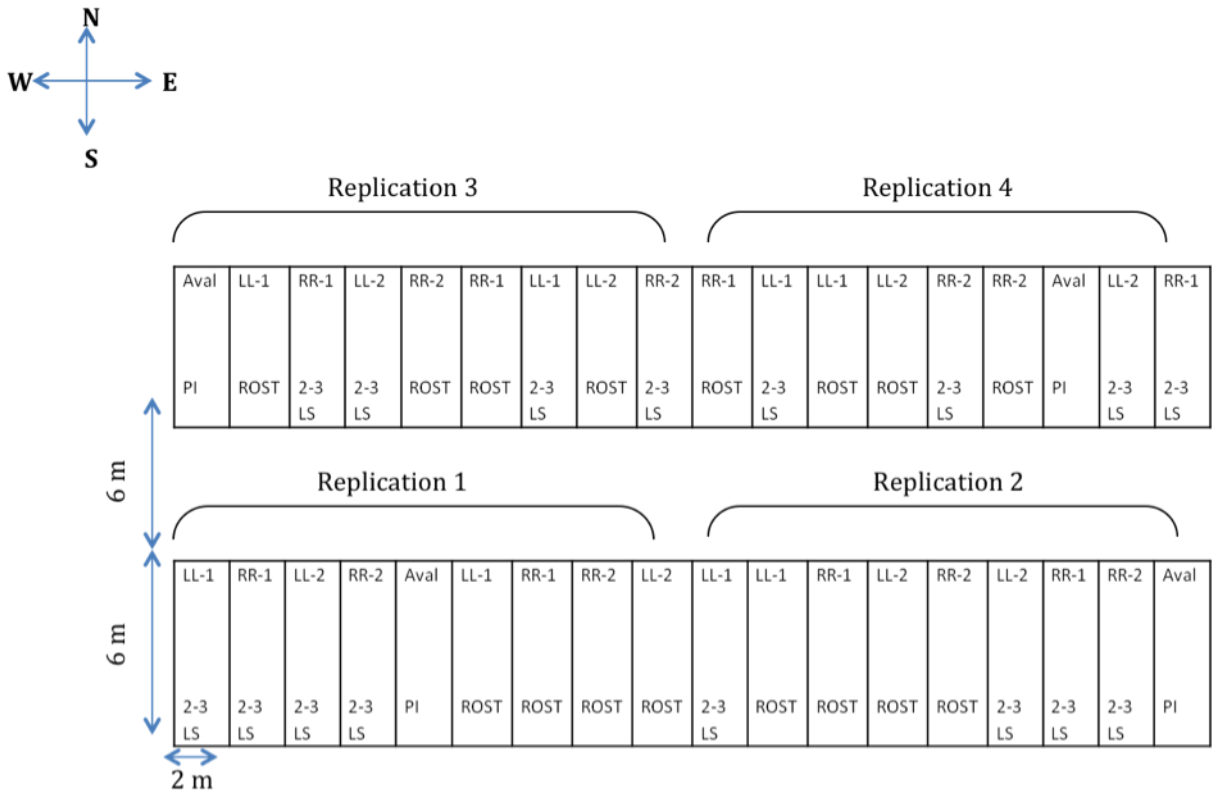

Figure 3.1 Field plot layouts at both locations in Quebec, as per RCBD

(RR-1 means roundup ready-variety 45H28, RR-2 means roundup ready -variety 45H29, LL-1 means liberty link- variety 5030, LL-2 means liberty link- variety 5040, 'Aval' means avalanche-conventional canola cultivar. In the figure PI represents pre-incorporated herbicide)

Figure 3.2 illustrates how HR and conventional canola cultivars were planted, herbicide applications timed, and what layers of soil were sampled. Unlike the HR cultivars, conventional canola was planted after trifluralin incorporation into the soil, and did not receive further applications during growth. Each treatment was replicated four times at both locations. Field plots were 6 m × 2 m size with 0.18 m row spacing. Canola was planted on May 31, 2011 at the Lods site, and May 30, 2011 at the Normandin site. Physico-chemical soil properties of both sites are presented in Table 3.1.

3.2 Determination of canola yield

Seed yield was determined from each experimental plot. Canola was harvested using a combine harvester (Figure 3.3). Harvesting was conducted when most of the seeds were mature and bore little or no green color. The combine harvester used for harvesting was thoroughly cleaned prior to entering each new plot. The yield of each plot was placed in a separate pre-weighed bag, and the seed weight was obtained by subtraction of the bag weight. Seeds were dried at room temperature in a well-ventilated room for a week before weighing. The seed weight in kilograms was multiplied by 833.3 [i.e., 10000 m^2 ÷ (2 m × 6 m)] to obtain yield in kg ha^{-1}.

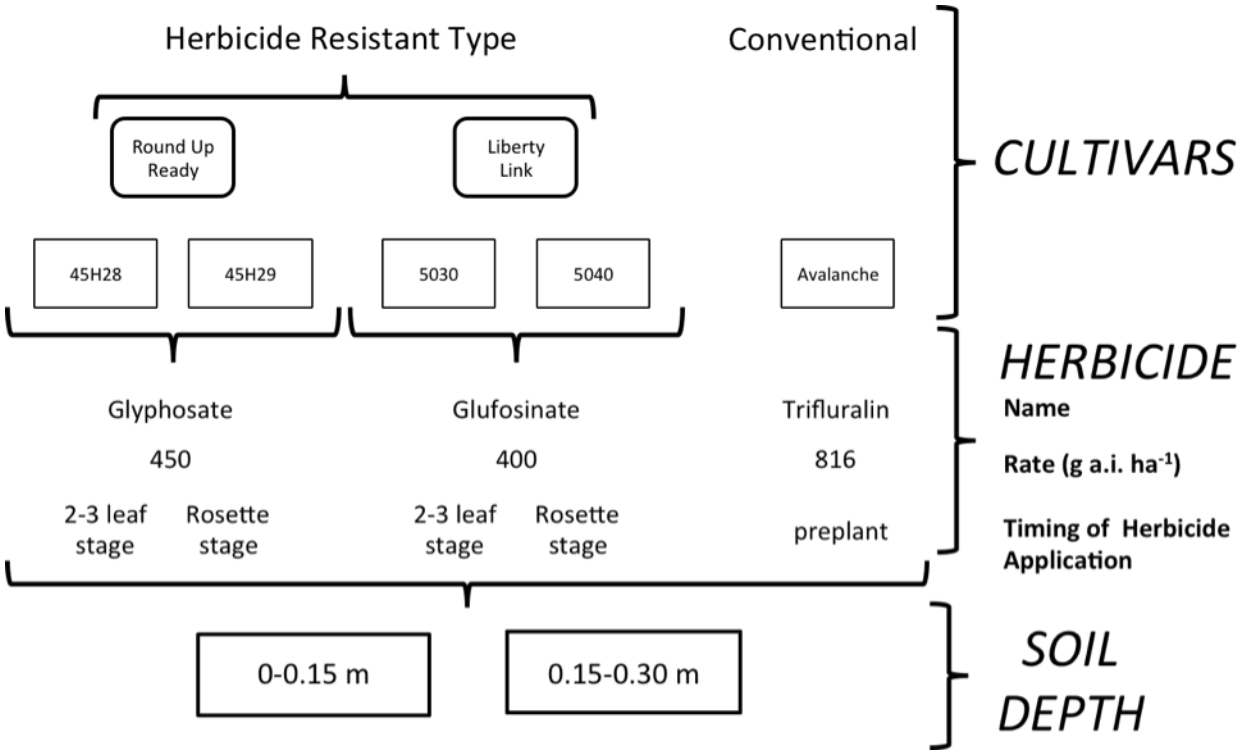

Figure: 3.2 Treatment assignments and sampling procedures for both field sites

Table 3.1: Physical and chemical properties of soils at Lods and Normandin sites

Soil Parameters	Lods Site	Normandin Site
Sand (%)	11	59
Silt (%)	64	33
Clay (%)	25	8
SOM (%)	3.97	4.70
pH	5.19	5.51
P (mg kg^{-1})	80.1	47.8
K (mg kg^{-1})	82	166
Ca (mg kg^{-1})	840	1450
Mg (mg kg^{-1})	100	173
Al (mg kg^{-1})	1260	1310
CEC (cmol kg^{-1})	6.06	9.18

Figure 3.3 Combine harvester harvesting canola crop at the Lods Research Farm

3.3 Herbicide applications

Herbicide application schedules were chosen based on the common practices of Quebec farmers to spray the canola crop at the 2-3 leaf or rosette stages. The stage at which herbicide applications were made was based on traditional Quebec farmers' practices in growing canola. Equal numbers of plots (*i.e*., 2) were assigned for the application at 2-3 leaf stage or at the rosette stage in each replication. Plots with Roundup Ready and Liberty Link cultivars were treated with glyphosate (450 g a.i. ha^{-1}) and glufosinate ammonium (400 g a.i. ha^{-1}), respectively. Conventional canola variety, Avalanche, received trifluralin at a rate of 816 g a.i. ha^{-1}. Glyphosate and glufosinate herbicides were sprayed as post-emergent herbicides, whereas trifluralin was applied a day before sowing the canola, as a pre-plant herbicide, incorporated to a depth of 50-60 mm to check its rapid volatilization (Grover *et al*., 1997). Glyphosate and glufosinate herbicide applications at either of

the crop growth stages (2-3 leaf stage or rosette stage) were applied in different plots. Soil sampling days during the study period are indicated in Table 3.2.

3.4 Soil sampling for herbicide residue analysis

Soil samples were collected from two depths (0-0.15 m and 0.15-0.30 m). Sampling was done with two augers, one for each soil depth, and these were thoroughly cleaned between each sampling activity. Samples were collected from two different points within each plot and then mixed together in a bag to get a representative sample from each depth. Soil samples from each plot were placed into Ziploc™ bags which were sealed and taken to the laboratory where they were stored at -20˚C until analysis. The time between soil sampling and storage varied between 6 and 8 hours. Soil samples were collected on the day prior to pesticide application, and 1, 7 and 20 days thereafter. The pre-application sampling served to determine what herbicidal residues, if any, were present in the field.

Table 3.2 Soil sampling days for the two field locations

Herbicide Name	Lods Location			Normandin Location		
	2-3 Leaf Stage	Rosette Stage	Time after Application (days)	2-3 Leaf Stage	Rosette Stage	Time after Application (days)
	Sampling Days	Sampling Days		Sampling Days	Sampling Days	
Glyphosate	21-Jun-11	08-Jul-11	1	22-Jun-11	29-Jun-11	1
	27-Jun-11	15-Jul-11	7	28-Jun-11	05-Jul-11	7
	10-Jul-11	28-Jul-11	20	11-Jul-11	18-Jul-11	20
Glufosinate	21-Jun-11	08-Jul-11	1	22-Jun-11	29-Jun-11	1
	27-Jun-11	15-Jul-11	7	28-Jun-11	05-Jul-11	7
	10-Jul-11	28-Jul-11	20	11-Jul-11	18-Jul-11	20
*Trifluralin	Pre-incorporated (Sampling-Days)		Time after Application (days)	Pre-incorporated (Sampling- Days)		Time after Application (days)
	01-Jun-11		1	01-Jun-11		1
	07-Jun-11		7	07-Jun-11		7
	20-Jun-11		20	20-Jun-11		20

* Trifluralin, a soil-incorporated pre-emergence herbicide, was not applied at 2-3 leaf stage or rosette stage

3.5 Extraction of herbicidal residues

3.5.1 Chemicals and apparatus

Glyphosate, aminomethyl phosphonic acid (AMPA) and glufosinate analytical standards (99% purity) were purchased from Sigma Aldrich (St. Louis, MO), whereas the trifluralin's analytical standard (99.9% purity) was purchased from Supelco (Bellefonte, PA). These were used in the preparation of stock solutions to run the calibration curves for each herbicide. A Microsorb-MV 100-5 Amino 250 × 4.6 mm column was purchased from Agilent Technologies (Santa Clara, CA) and a Nova Pak C18, 300 × 3.9 mm column from Waters (Milford, MA). All solvents and other chemicals used in this study were HPLC-grade. The water used during solution preparation and analysis was obtained from a Milli-Q (Billerica, MA) system (resistivity > 18 MΩ cm).

3.5.2 Soil extraction for glyphosate

The extraction method of Druart *et al*. (2011) was followed, with the exception of the solvent used. They used 0.1 M NaOH as the extraction solvent but in our extraction procedure, 0.6 M KOH was used, as described by Ibáñez *et al*. (2005).

Air-dried soil samples (5 g) were homogenized in 10 ml of 0.5 M KOH in a 50 mL centrifuge tube, and extracted by shaking (at 110 rpm) on a mechanical shaker for 1 h, followed by standing for 1.5 h, and being shaken again for 1 h. Timings for the extraction procedure were adapted from the method proposed by Druart *et al*. (2011). The extracts were then centrifuged at 2054 G force for 20 min. The alkaline supernatant was decanted and neutralized by adding drops of 5M and 0.5M HCl until pH 7(0.5M molarity of HCl was used when pH nearly reach 7). This neutralization step was necessary to carry out derivatization of the compounds (Ibáñez *et al*., 2005).

3.5.3 Soil extraction for glufosinate

The extraction for glufosinate ammonium followed the same procedure, as for glyphosate, except that distilled water was used as the extractant, as it allowed better recovery of this compound from soil (Druart *et al*., 2011). The supernatant was decanted and neutralized (only those samples which showed minor acidic pH) by adding drops of 0.5M KOH until pH 7 was reached. This neutralization step was necessary to carry out derivatization of the compounds.

3.5.4 Soil extraction for trifluralin

A 5 g soil sample was placed in a 50 mL centrifuge tube and extracted with 20 mL acetonitrile by shaking in a vortex for 1 min, followed by 10 min of sonication. Sonication has been shown to provide a better contact between solvent and solids, resulting in good recoveries (Babić *et al*., 1998; Poole *et al*., 1990). This extraction procedure was repeated twice with a further 15 ml acetonitrile on each occasion. Thereafter, the combined extract was centrifuged at 2683 G force for 10 min, and the supernatant was concentrated by evaporating down to 1 mL under a nitrogen stream. The extract was then filtered through a 0.22 µm syringe filter (Millipore) and transferred into HPLC vials for analysis.

3.6 Derivatization of glyphosate and glufosinate

Extracted samples for glyphosate and glufosinate were derivatized, according to the method described by Le Bot *et al*., (2002). The lack of a chromophore or fluorophore in these compounds necessitated derivatization (Stalikas and Konidari, 2001).

Derivatization was performed by adding 0.5 mL of 0.05M borate buffer (pH ≈ 9) and 0.5 mL of FMOC-Cl [Chloroformic acid 9*H*-fluoren-9-ylmethyl ester] solution (prepared by adding

10 mg FMOC-Cl to 10 mL of acetonitrile) to 3 mL of the sample solution. The resulting mixture was agitated on a mechanical shaker for 1 h at room temperature to allow the reaction to complete. Within 1 hour, 98% of the reaction was completed, as explained by Le Bot *et al*. (2002). The excess FMOC-Cl reagent was removed by performing liquid-liquid extraction with 2 ml of diethyl ether with the help of separatory funnel and keeping the aqueous phase. Derivatized samples were filtered with syringe filters (0.45 µm) directly into HPLC vials for analysis.

3.7 Analysis of soil extracts

3.7.1 Glyphosate and glufosinate ammonium Analysis

LC analysis was performed according to the "Application Note of Agilent Technologies for the detection of Glyphosate" using a Varian ProStar HPLC system (Model 242), equipped with a Varian ProStar 363 fluorescence detector and Varian ProStar 410 auto sampler with the a Galaxie computer data acquisition system. Compound separation was obtained on a Microsorb-MV 100-5 Amino 250 mm × 4.6 mm column. Separation of components employed a mobile phase of phosphate buffer (prepared in ultrapure water obtained from Milli-Q system) (pH 5.8) and acetonitrile (in the ratio of 55:45), with an isocratic program. The flow rate was 1 mL min^{-1} with the injection volume of 50 µL. The detection response of each derivatized sample was obtained at excitation and emission wavelengths of 260 nm and 310 nm, respectively. Limit of detection (LOD) of the instrument was 0.1 µg/L and limit of quantification (LOQ) was 0.3 µg/L.

The concentration of glyphosate and glufosinate in the samples was determined by comparing the area of glyphosate peak in the sample chromatogram to that of a glyphosate and glufosinate standard solution chromatograms, through an external calibration curve. The herbicide concentrations were ultimately expressed as µg kg^{-1} of soil.

3.7.2 Trifluralin Analysis

Analysis of trifluralin was carried out with an HPLC instrument (Agilent 1100 series) equipped with an auto sampler and UV detector. Response was quantified with the HPLC's Chem-Station software. The response of the detector was obtained at 235 nm. Separations were obtained with a reverse-phase column (Nova Pak C18, 300 × 3.9 mm) and the mobile phase was acetonitrile and water in a ratio of 70:30, with a flow rate of 1 mL min^{-1}. The injection volume was set at 20 µl. The mobile phase was filtered and degassed prior to use. Limit of detection of the instrument was 0.04 mg/L and limit of quantification was 0.1 mg/L. The trifluralin concentrations in the soil samples were determined by comparing the area of trifluralin peak in the sample chromatogram with the areas of trifluralin standard solution chromatograms by quantifying with the external calibration curve.

3.8 Data analysis

Glyphosate, glufosinate and trifluralin herbicide degradation was described by first order kinetics:

$$C(t) = C_0 e^{-kt} \quad (3.1)$$

where

k degradation rate constant (day^{-1})

t time after herbicide application (days)

$C(t)$ concentration of the herbicide residue at time 't'

C_0 initial herbicidal residue concentration at time $t = 1$.

The half-life (DT_{50}) values for these herbicides were calculated using the following equation:

$$DT_{50} = \frac{ln\ (2)}{k} \tag{3.2}$$

Where

DT_{50} is the time required to degrade 50% of the herbicide concentration initially present (Mamy et al., 2005).

The levels of pesticide were adjusted according to the rate of herbicide recovery, and subjected to repeated measures ANOVA using the PROC GLM procedure in SAS v. 9.3 (SAS Institute Inc., 2011). The concentrations from the conventional canola production system was not analyzed statistically alongside those from the HR canola cultivars, given the different treatment factors; ANOVA was applied to the herbicide resistant cultivars only.

CHAPTER 4

Results and Discussion

4.1 Comparison of HR and conventional canola variety yields

Canola yields assessed at the Lods site for different HR varieties and different pesticide application times are shown in Figure 4.1, as is the yield for the conventional cultivar 'Avalanche'. Neither HR variety nor stage at which the herbicide was applied had a significant effect on yield ($P > 0.05$; Table 4.1)

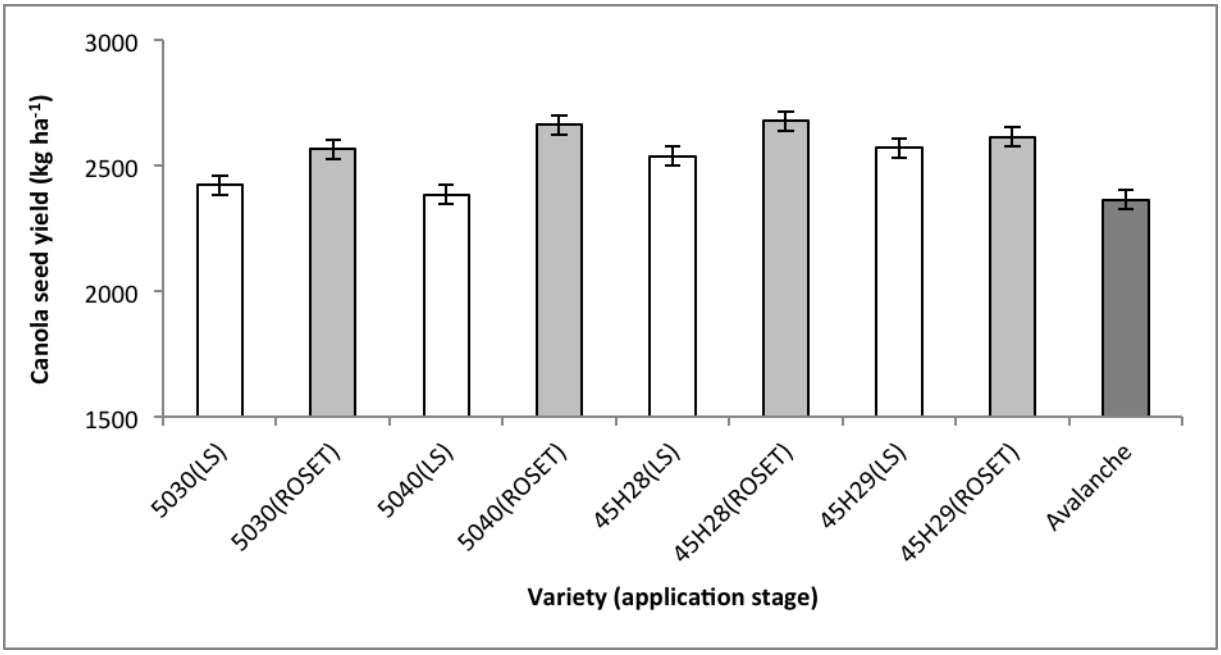

Figure 4.1: Yield comparisons of glyphosate-resistant varieties (45H28 and 45H29;), glufosinate resistant varieties (5030 and 5040;) and conventional variety (Avalanche;) of canola at the Lods site (LS= 2-3 leaf stage; ROSET= rosette stage). Error bars represent ± one standard error.

Table 4.1: Analysis of variance for canola seed yield

Dependent Variable: Yield					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**P value**
Variety	69991.844	3	23330.615	0.146	0.931^{ns}
Stage	182559.031	1	182559.031	1.141	0.296^{ns}
Variety × Stage	54745.594	3	18248.531	0.114	0.951^{ns}
Error	3841433.250	24	160059.719		

ns: not significant

The 'Avalanche' variety of conventional canola was not included in the analysis of variance due to lack of application stages and further sub varieties of conventional canola as exist for the HR canola varieties. Hence statistical analysis could not be performed due to this inconsistency. While the differences were not statistically significant, the Roundup ready variety, 45H28 produced the highest yield. Compared to the HR varieties, the conventional cultivar 'Avalanche' produced the lowest yield (7.4 % less than the HR varieties). O'Donovan *et al*. (2006) had also found that glyphosate resistant canola systems were profitable and gave better yield than traditional canola cultivars.

4.2 Calibration curves and herbicides recovery

In case of glyphosate, the linear response of the external calibration curve was obtained by using seven concentrations (0.3, 0.4, 0.5, 1, 5, 10, 20 and 50 µg L^{-1}) of glyphosate (R^2 = 0.9886; Figure 4.2). An external calibration was also run for glufosinate with six concentrations (0.3, 0.4, 0.5, 1, 10 and 20 µg L^{-1}) and a strong linear correlation was obtained (R^2 = 0.999; Figure 4.3). The concentration 50 µg L^{-1} was not used in case of glufosinate since it was giving a flattop peak due to high concentration, whose area was difficult to quantify accurately. Fortunately concentrations in all the soil samples were less than this value. The herbicide concentrations were ultimately expressed as µg kg^{-1} of soil.

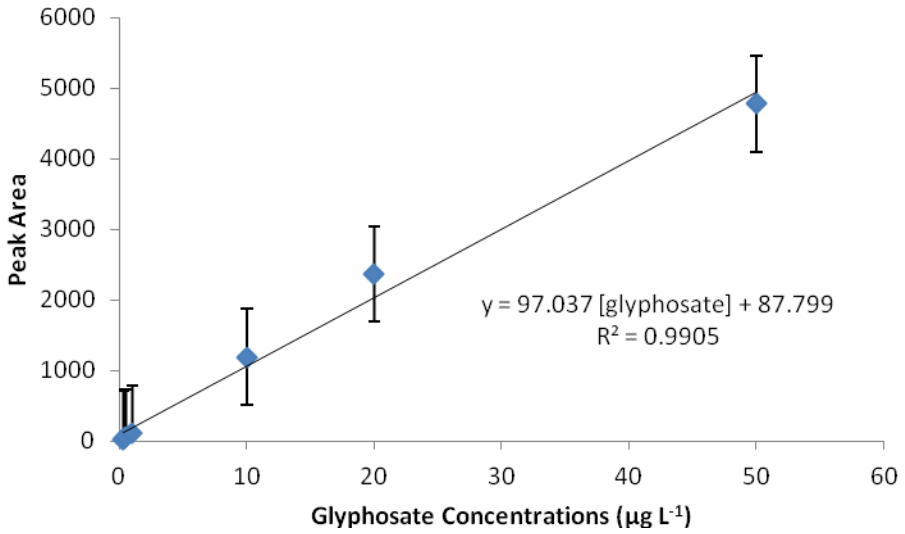

Figure: 4.2 Calibration curve for glyphosate

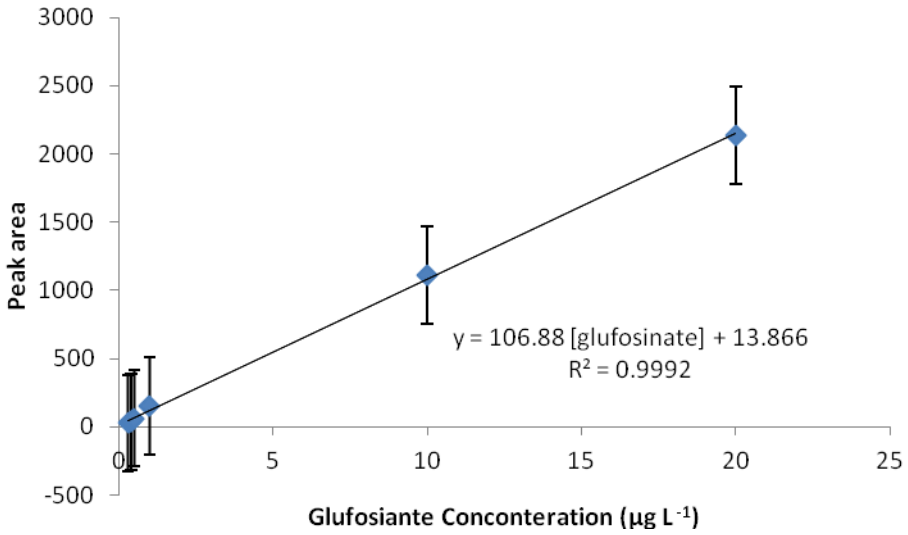

Figure 4.3: Calibration curve for glufosinate

The calibration curves for all three herbicides showed good linearity and correlation coefficients (R^2), in the range of 0.99-1.00. Calibration of the trifluralin was performed over seven concentrations (0.1, 0.2, 0.4, 0.5, 1, 5 and 10 mg L^{-1}). A good linear response (R^2 = 1; Figure 4.4) was obtained.

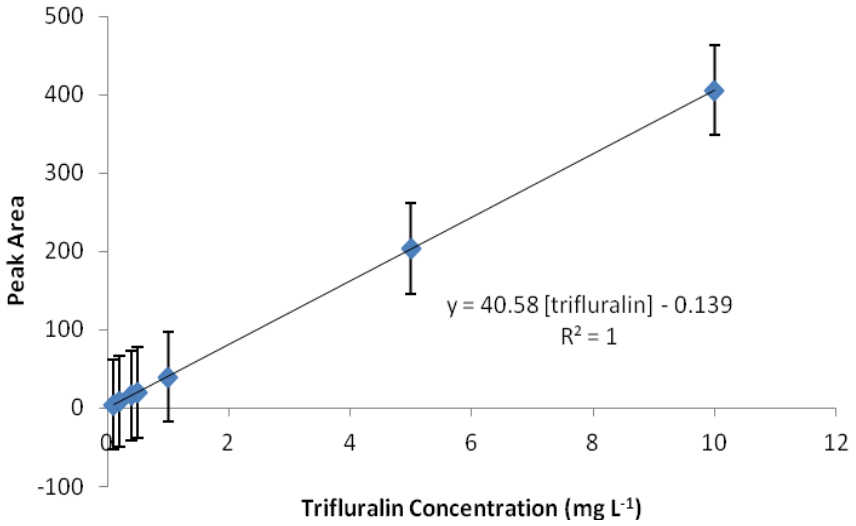

Figure 4.4: Calibration curve for trifluralin

Glyphosate's recovery from soil samples was 40% with a relative standard deviation (RSD) of 2.1% while the recovery of glufosinate from soil samples was 50% with an RSD 1.66%. Druart *et al*. (2011) obtained recovery values of 27.5% and 42.6% for glyphosate and glufosinate.

4.3 Screening for pesticide residues present prior to in-study applications

Soil samples collected from both field locations, prior to herbicide application, were analyzed, using the same methods of extraction and analysis, for their glyphosate, glufosinate ammonium and trifluralin residues. None of the samples was found to have detectable level of these herbicides.

4.4 Lods field site

The physical and chemical properties of the Macdonald field soil were presented in Table 3.1. It was a silt loam soil, with a 3.97 % organic matter content and pH of 5.19.

4.4.1 Repeated measures analysis

Table 4.2 shows the results of multivariate (repeated measures) analysis, using the Wilk's test, which was conducted to test the significance of within-subjects (time) main and interaction effects in influencing herbicide concentrations in the 0-0.15 m layer of soil. The nearly all-zero pesticide concentration values for the 0.15-0.30 m soil layer meant the data did not conform to the normality assumption, thus precluding analysis of this layer, or of any depth effects. The time factor (number of days after herbicide application) was significant ($P \leq 0.0001$), indicating that the herbicide concentration changed with the passage of time. Similarly, the results of multivariate tests (Table 4.2, rows 2 and 3) show that both time × cultivar and time × stage

interactions were significant ($P \leq 0.001$), implying that the change in mean herbicide concentration over time depends on cultivar type as well as plant growth stage selected for herbicide application. The other interaction effects were not significant ($P > 0.05$; Table 4.2, rows 4-6). The results of univariate tests (not shown) to assess significance of within-subjects effects (time main effect) yielded similar findings.

Table 4.2: Repeated measures analysis for within-subjects main and interaction effects at the Lods site

S. No.	Source	Wilk's Lambda	F Value	p-value
1.	Time	0.03958320	279.03	<0.0001***
2.	Time × Cultivar	0.10067215	102.73	<0.0001***
3.	Time × Stage	0.52717416	10.31	0.0006***
4.	Time × Variety(Cultivar)	0.90481029	0.59	0.6717^{ns}
5.	Time × Cultivar×Stage	0.88351652	1.52	0.2407^{ns}
6.	Time×Stage×Variety(Cultivar)	0.83906583	1.05	0.3898^{ns}

*** significant at 0.1% level, ns: not significant

The significance of between-subjects main and interaction effects for topsoil (0-0.15 m) herbicide concentrations are presented in Table 4.3. Both cultivar type (Roundup Ready *vs.* Liberty Link) and stage of herbicide application (2-3 leaf *vs.* rosette) had a statistically significant effect ($P \leq 0.0001$ and $P \leq 0.01$, respectively) on herbicide concentrations in the soil, implying that both these factors influenced herbicide concentration. The variety effect (nested in Cultivar) was not significant ($P > 0.05$), indicating that no difference existed between varieties 45H28 & 45H29 (Roundup Ready), or between varieties 5030 & 5040 (Liberty Link). The interaction effects of cultivar × stage and stage × variety (cultivar) were non-significant ($P > 0.05$; Table 4.3 - rows 4, 5). This shows that there are no interactions between subjects in this study.

Table 4.3: Repeated measures of analysis between-subjects main and interaction effects at the Lods site

S. No.	Source	DF	Mean Square	F-Value	p-value
1.	Cultivar	1	40546.79	103.34	<0.0001***
2.	Stage	1	3226.193	8.22	0.0085**
3.	Variety (Cultivar)	2	279.3763	0.71	0.5007 ^{ns}
4.	Cultivar × Stage	1	237.4475	0.61	0.4442 ^{ns}
5.	Stage × Variety (Cultivar)	2	442.6684	1.13	0.3402 ^{ns}

** significant at 1% level, *** significant at the 0.1% level
ns: non significant

4.4.2 Herbicide Persistence at Lods site

Soil samples were analyzed for residual persistence of the three herbicides. Given that sampling time was significant (Table 4.2) and considering that a significant difference by cultivar type was to be expected, since each of two cultivar types received different herbicides, at different rates, a simple ANOVA analysis was applied to each sampling time (1, 7 and 20 days after herbicide application), with the lower soil depth (0.15-0.30 m) again excluded as being nearly all-zero. Notwithstanding this data being excluded from the ANOVA, it is clear (Figures 4.5 and 4.6) that 1 and 7 days after applications, herbicide levels in the upper topsoil (0-0.15 m) were consistently higher (at least 8-fold) than those in the lower topsoil (0.15-0.30 m).

The results of classical ANOVAs for the three sampling times are presented in Table 4.4. As expected, the effect of cultivar (i.e. pesticide applied) was significant, with the topsoil levels of glyphosate being significantly lower ($P \leq 0.0001$) than those of glufosinate at each sampling date. The stage of herbicide application was a significant ($P \leq 0.0001$) factor for the sampling at

7 days after application, but not at 1 or 20 days (Table 4.4.) For day 7 sampling, soil concentrations of glyphosate were 42% lower following the rosette stage application, than after a 2-3 leaf stage application, while this reduction was 23% for glufosinate. At day 1 after application, these decreases were 48% and 4.4% respectively, i.e., showing the same trend as after 7 days, while being close to, but not significant ($P = 0.1029$). Herbicides residue levels were not influenced by variety, cultivar × stage interaction and stage × variety interactions. Consequently, mean pesticide levels across varieties (n = 2 var × 4 reps = 8) were calculated within Roundup Ready and Liberty Link cultivars, and presented in Figures 4.5 and 4.6.

4.4.2.1 Glyphosate and glufosinate residues in soil for the 2-3 leaf stage application

As expected, following application at the 2-3 leaf stage, maximum concentrations of the glyphosate and glufosinate (Figure 4.5) were present at 0-0.15 m top soil layer. Degradation was faster for glufosinate ammonium than glyphosate, *i.e.*, glyphosate would persist longer than glufosinate ammonium. At 7 days after the glyphosate and glufosinate ammonium were applied, 33.16% and 37.54% of initial glyphosate and glufosinate ammonium amounts were lost (Figure 4.7). A longer persistence of glyphosate, compared to glufosinate ammonium, has been reported in other soils (Accinelli *et al*., 2004; Laitinen *et al*., 2006). In the present study, glufosinate dropped to undetectable levels, 20 days after 2-3 leaf stage herbicide application. Similar results were reported by Laitinen *et al*. (2006) while monitoring the fate of glufosinate along with four other herbicides. They observed DT_{90} values for glufosinate of 10-30 days at 20˚C. However, at the Lods field site, average daily temperature exceeded 20˚C (mentioned by Laitinen *et al*., 2006) by 1.07˚C. Higher soil temperatures can lead to a faster degradation process (El Sebaï *et al*., 2011).

On the other hand, glyphosate was still present at detectable levels, 20 days after application at the 2-3 leaf stage (Figure 4.5). It should be noted that zero concentration values after 20 days may not represent the absence of residues. The herbicide extraction efficiencies were low (50% for glufosinate with RSD 1.66% and 40% for glyphosate with RSD, 2.1%) for our soils. Secondly, the residues remaining might be present at levels below the instrument's detection limit.

Both herbicides were absent at the lower depth (0.15-0.30 m) after 1 and 20 days of herbicide treatment (Figures 4.5 and 4.6). However, a negligible amount of both herbicides was present 7 days after application in the lower depth (0.15-0.30 m). This vertical movement of glufosinate ammonium and glyphosate can be explained by the 25.2 mm cumulative rainfall which occurred within 7 days of the herbicide application (Figure 4.5). The mobility and leaching of glyphosate has been reviewed comprehensively by Vereecken (2005) who broadly discussed the movement of glyphosate in laboratory-, lysimeter- and field-scale experiments, noting in all three cases that these highly sorbing herbicides may move vertically with preferential flow/transport in the event of rainfall after the application of herbicide. Similar results were also reported regarding glyphosate movement (Borggaard and Gimsing, 2008).

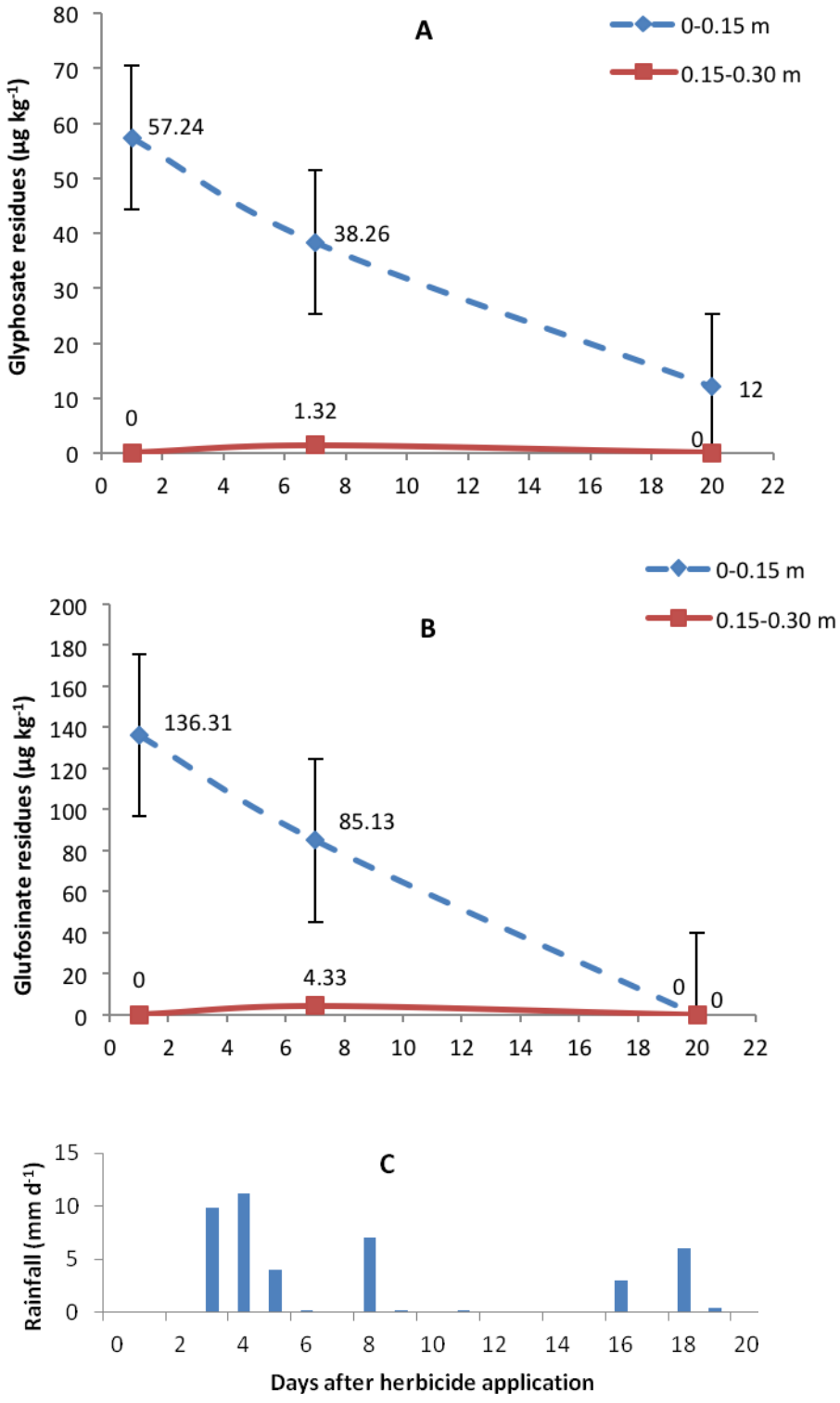

Figure 4.5: Residual levels of pesticides applied at 2-3 leaf stage in soil at two depths (0-0.15 m and 0.15-0.30 m), Lods site. A. Glyphosate, B. Glufosinate, C. Daily rainfall

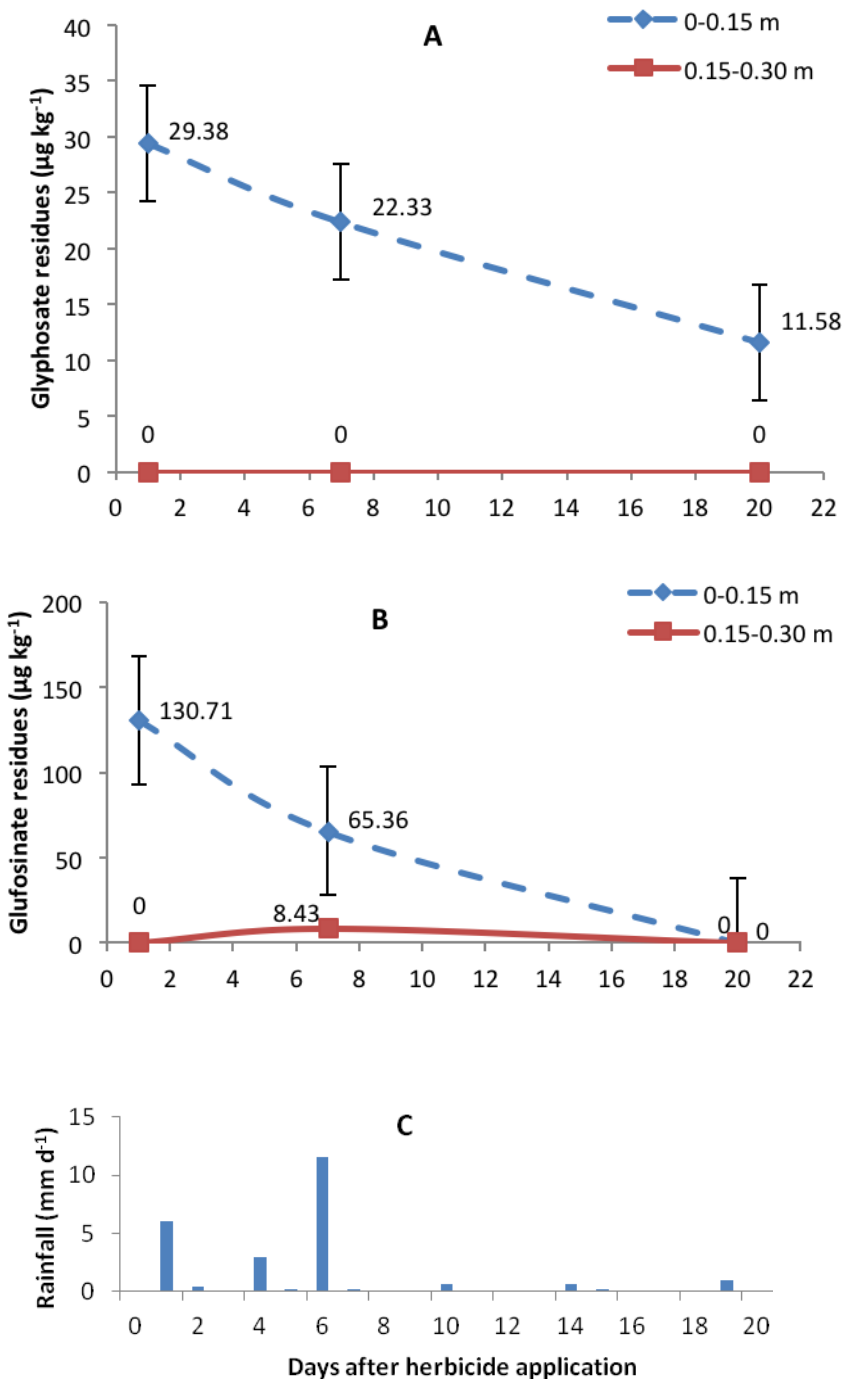

Figure 4.6: Residual levels of herbicides applied at the rosette stage in soil at two depths (0-0.15 m and 0.15-0.30 m), at the Lods site. A. Glyphosate, B. Glufosinate, C. Daily rainfall

Table: 4.4: Analysis of variance of herbicide concentrations in the top 0.15 m of soil for sampling days 1, 7 and 20 days after application, Lods site

Source	DF	Mean Square	F value	P value
Day after herbicide application				
Cultivar (RR *vs.* LL)	1	65061.2628	83.73	<0.0001***
Stage	1	2234.4612	2.88	0.1029^{ns}
Variety	2	453.7914	0.58	0.5654^{ns}
Cultivar × Stage	1	995.0260	1.28	0.2690^{ns}
Stage × Variety	2	874.7365	1.13	0.3409^{ns}
7 days after herbicide application				
Cultivar (RR *vs.* LL)	1	16158.6264	154.58	<0.0001***
Stage	1	2547.1953	24.37	<0.0001***
Variety	2	97.6216	0.93	0.4069^{ns}
Cultivar × Stage	1	30.1864	0.29	0.5960^{ns}
Stage × Variety	2	128.4210	1.23	0.3105^{ns}
20 days after herbicide application				
Cultivar (RR *vs.* LL)	1	1116.7538	46.79	<0.0001***
Stage	1	0.4095	0.02	0.8969^{ns}
Variety	2	5.1842	0.22	0.8063^{ns}
Cultivar × Stage	1	0.4095	0.02	0.8969^{ns}
Stage × Variety	2	3.2896	0.14	0.8719^{ns}

*** significant at 0.1% level, ns: non-significant

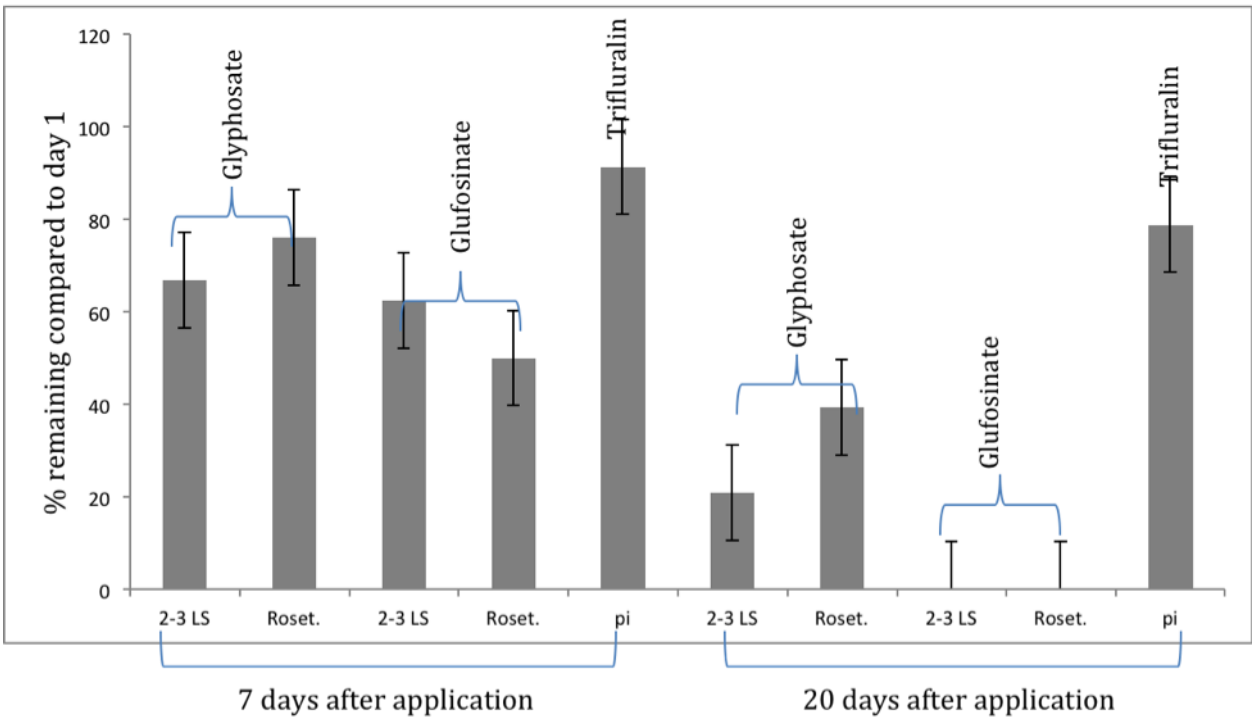

Figure 4.7: Percent herbicide residues remaining 7 and 20 days after application compared to herbicide residues present one day after application. Data for the Lods site, three herbicides and both stages of herbicide application, where applicable. Error bars show standard errors. In this figure, '2-3 LS' represents 2-3 leaf-stage, 'Roset' represents rosette and 'pi' stands for pre-incorporated stage of herbicide application.

On the other hand, the high water solubility of glufosinate ammonium and its low adsorption may increase the risk of glufosinate movement to a deeper depth; hence, its mobility can be greater than that of glyphosate (Laitinen *et al*., 2008). However, despite of its susceptibility to leaching, very low risk of contamination to water bodies could be expected due to its high degradation rate and shorter half- life (Siimes *et al*., 2006). This lesser risk of contamination with glufosinate *vs.* glyphosate is illustrated at 20 days after application (Figure 4.7) where the percent

remaining, compared to 1 day after application, is much lower for glufosinate than glyphosate. A similar trend was also observed for 7 days after application data.

4.4.2.2 Glyphosate and glufosinate residues in soil for the rosette stage application

Glyphosate and glufosinate residues in soil following application at the rosette stage are presented in Figure 4.6**.** At all three sampling dates (1, 7 and 20 days after application) glyphosate and glufosinate residual concentrations after application at the rosette stage were lower than those following application at the 2-3 leaf stage (Figure 4.6 *vs.* Figure 4.5). This might be attributable to interception of spraying mist by the larger canopy of the canola plants at the rosette stage than at the 2-3 leaf stage. Compared to day 1 after application, 24% of glyphosate and 50% of glufosinate ammonium were dissipated at 7 days, and 60.58% glyphosate and ~100% of glufosinate at 20 days after application (Figure 4.7). Thus, as after the 2-3 leaf stage application, glyphosate showed a greater persistence after being applied at rosette stage than did glufosinate ammonium. This concurs with other reports (Accinelli *et al*., 2004; Laitinen *et al*., 2006), although these studies were not specific to the crop stage at which herbicide applications were made.

No leaching or vertical movement of glyphosate from topsoil (0-0.15 m) to a deeper soil depth (0.15-0.30 m) was found (Figure 4.6). However, glufosinate ammonium did show some leaching or downward movement. Seven days after glufosinate ammonium application at the rosette stage, roughly 6% of glufosinate had leached down to the deeper layer, probably as a result of the 21.4 mm of rainfall which occurred between herbicide application and soil sampling 7 days thereafter at rosette stage. On the other hand, glyphosate was not transported into deeper depths despite this rainfall amount. Laitinen *et al*. (2006) also described low mobility of

glyphosate due to higher K_f (adsorption coefficient) values and high mobility of glufosinate ammonium because of low K_f values in sandy and clay loam soil. Glyphosate was found to have moved below 0.15 m when the crop was sprayed at the 2-3 leaf stage (Figure 4.5), but not when sprayed at the rosette stage. This movement of glyphosate, though it occurred under roughly the same intensity of rainfall, might be attributable to interception of the material sprayed by the larger rosette canopy, and greater uptake of percolated water from the soil, given the greater plant size. Moreover, the average temperature of the week following glyphosate application at the 2-3 leaf stage was lower than average temperature in the week after the rosette stage application (Appendix A-3). Hence, higher temperature at the rosette stage may have caused greater evapotranspiration and less percolation of water into deeper soil layers and also higher temperature can cause higher degradation process which could hinder the leaching process.

The average concentrations of glyphosate in topsoil (0-0.15 m) were low, ranging from 57 to 11 µg/kg of soil (Figures 4.5 and 4.6). These values were calculated based on 0 to 0.15 m soil depth which may not be the best way to represent results if glyphosate leaching was restricted to only a few millimeters, e.g. 2 mm reported by Haney *et al*. (2000). The 2-mm penetration depth can be expected in a silt loam soil, such as the one at the Lods site because the soil has 25% clay content. The high clay content can lead to higher sorption of glyphosate (Colombo and Masini, 2011) in the topsoil. Therefore, glyphosate concentrations in the topsoil were recalculated, following Haney *et al*. (2000), by assuming that glyphosate remained in the top 2 mm of soil. The recalculated glyphosate concentrations are given in Figure 4.9. The steps involved in recalculations are described below:

Measured bulk density of soil = 1460 kg m^{-3}

Hence, 1 m^3 of soil weighs.....1460 kg

Concentration of glyphosate obtained one day after application (for example) from 0.15 m^3 (0.15 m x 1 m x 1 m) soil slice = 57.24 µg kg^{-1}

Amount of glyphosate in 0.15 m^3 = 57.24 x (1460 x 0.15) µg

If all glyphosate was contained in the top 2 mm of soil, the concentration of glyphosate in 2 mm soil depth would be 57.24 x (1460 x 0.15)/(0.002 x 1460) µg kg^{-1} or 4293 µg kg^{-1}

The denominator (0.002 x 1460) is the dry weight of soil in 2 mm (0.002 m^3) soil slice.

Many authors have confirmed the high sorption of glyphosate (Ulén *et al*., 2012; Keshteli et al., 2011; Colombo and Masini, 2011). The high sorption of glyphosate in our soil was confirmed by calculating adsorption coefficient value by carrying out a batch sorption study. Adsorption isotherm was appraised by using Freundlich's adsorption equation:

$$\mathbf{S = K_f \, x \, C^n_f}$$

Where S is the amount of pesticide adsorbed in mg Kg^{-1} of the adsorbent; C is the equilibrium solution concentration (mg L^{-1}); K_f is the adsorption coefficient and n_f is the Freundlich exponent. Adsorption isotherm of glyphosate herbicide on Lods site's soil is shown in Figure 4.8. The high Freundlich coefficient value (63.66) for our soil is in line with other studies (Keshteli et al., 2011; Laitinen et al., 2006; Laitinen et al., 2008), whereas n_f value (0.3034) suggests the nonlinearity and strong concentration dependence of adsorption of the soil. Moreover, adsorption of glyphosate in soil also increases with high iron and aluminum content (Wang et al., 2005; Gerritse et al., 1996).

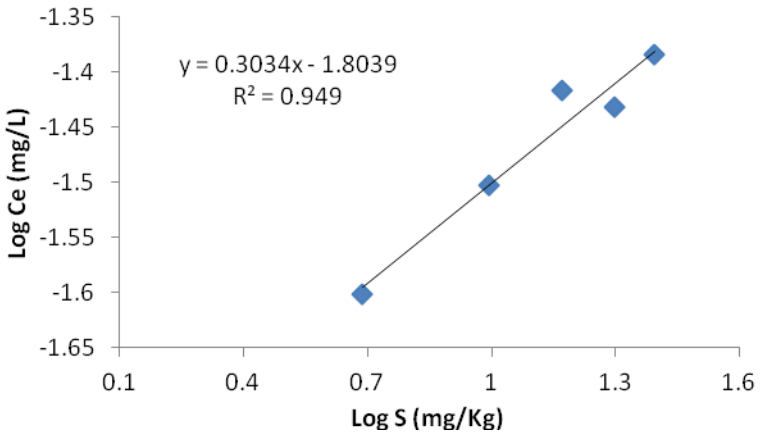

Figure 4.8: Adsorption isotherm of glyphosate herbicide on Lods site's soil.

The silt loam soil at the Lods site also has high clay and very high aluminium content. The average aluminium content in agricultural soils should be between 500-800 mg kg^{-1} (information available at www.holmestead.ca/chemtrails/soiltest.html) but the soil at the Lods site contained 1260 mg kg^{-1} (Table 3.1).

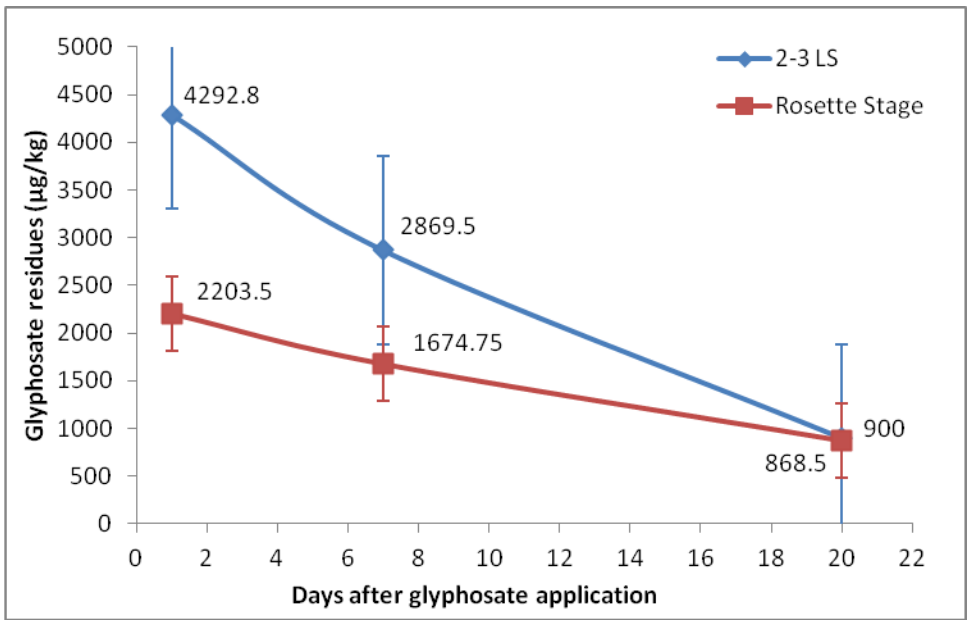

Figure 4.9: Residual concentrations of glyphosate calculated from 2 mm soil depth at 2-3 LS and rosette stage.

From Figure 4.9, if the glyphosate movement was restricted to the top 2 mm soil depth, this can pose great environmental risks. With any heavy rainfall event, the surface runoff from such canola fields may contain significantly higher concentrations of glyphosate due to soil erosion and/or high solubility of glyphosate in water (10.5 g L^{-1} as per Table 2.1). It is very likely that glyphosate concentrations in runoff water will be way higher than glyphosate's maximum allowable limit for drinking water of 280 µg L^{-1} and the allowable limit for the protection of aquatic life of 65 µg L^{-1}, as per the Canadian water quality guidelines (CCME, 2012). This needs to be looked into more carefully in future studies.

4.4.2.3 Residual concentrations of trifluralin in soils studied at the Lods site

Trifluralin (a pre-emergent herbicide) was applied to a conventional canola variety (Avalanche) to control broadleaf weeds. The use of trifluralin has become questionable due to its longer persistence in the environment (Triantafyllidis *et al*., 2010). The day after trifluralin application, 673.89 µg kg^{-1} of this herbicide was detected in the 0-0.15 m soil depth. The dissipation of trifluralin residues with the passage of time showed 8.73% and 21.20% losses, respectively, 7 and 20 days after application, compared to levels one day after application (Figure 4.7). Kim and Feagley (2002) also found that most of the trifluralin remained in the top 0.15 m of soil. Our sampling plan extended only to 20 days; however, Triantafyllidis et al. (2010) studied dissipation of trifluralin for the period of 150 days and observed that first phase (20-25 days) of dissipation was faster than the second phase (25-150 days).

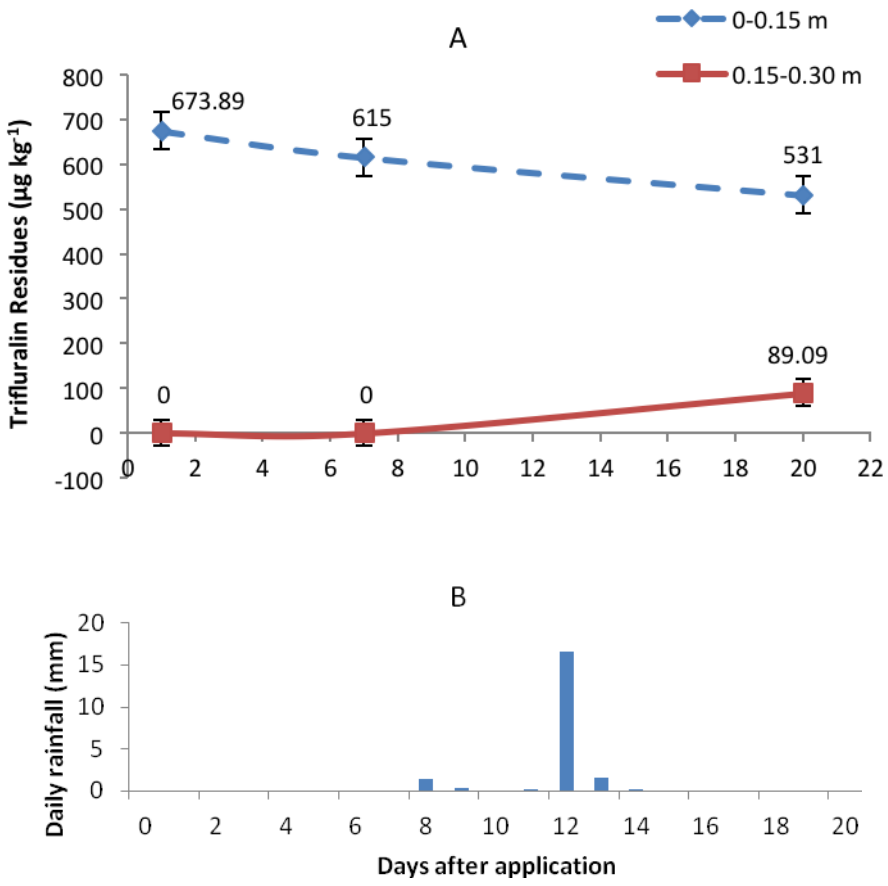

Figure 4.10: Residual levels of trifluralin in soil at two depths (0-0.15 m and 0.15-0.30 m) at the Lods site. A.Trifluralin B. Daily rainfall

In the lower depth of 0.15-0.30 m, no residues were found after 1 or 7 days of trifluralin application, while after 20 days, 89.09 µg kg^{-1} of this herbicide was detected (Figure 4.10A), corresponding to 13% of the amount observed at the day after trifluralin application in the top soil. The entire week following the herbicide application was dry, but the second week saw 20.4 mm of rainfall (Figure 4.10B). which could contribute in leaching process from 0.05-0.15 m trifluralin movement. Kim and Feagley (2002), studying the leaching of trifluralin in a clay loam soil in Louisiana, found that high adsorption of trifluralin caused minimum to negligible leaching

to deeper soil layers. However still they found leaching of trifluralin upto 0.6 m depth although approximate 94% trifluralin were present in 0-0.15 m soil-surface.

Figure 4.7 shows the overall dissipation/persistence behavior of all three herbicides with respect to soil residual levels 1 day after herbicide application. Twenty days after herbicide application, topsoil (0-0.15 m) glyphosate and glufosinate levels had declined 60-80% and ~100%, respectively, compared to only 30% for trifluralin. Herbicide persistence at the Lods site followed the order: trifluralin > glyphosate > glufosinate.

4.5 Normandin Site

The physical and chemical properties of the soil from Normandin were presented in Table 3.1. The Normandin site has rather different physico-chemical properties than the soil at the Lods site. The Normandin site has a sandy loam soil, with higher organic matter content, pH and CEC than the Lods site. The soil samples from Normandin were similarly analyzed for glyphosate, glufosinate and trifluralin 1, 7 and 20 days after herbicide application.

4.5.1 Statistical analysis for the experimental data from the Normandin site

Table 4.5 shows the results of multivariate (repeated measures) analysis conducted to test the significance of within-subjects main (time main effects) and interaction effects on herbicide concentrations in soil. The nearly all-zero pesticide concentration values for the 0.15-0.30 soil layer meant the data did not conform to the normality assumption, thus precluding analysis of this layer, or of any depth effects.

Similarly to the Lods site, the time factor (number of days after herbicide application) was significant ($P \leq 0.0001$), indicating that the herbicide concentration changed with the

passage of time (Table 4.5, row 1). While the time × cultivar is significant ($P \leq 0.0001$), as it was at the Lods site, unlike the Lods site, the time × stage interaction is not significant ($P > 0.05$), implying that the change in mean herbicide concentration over time depends on cultivar type but not on plant growth stage (Table 4.5, rows 2 and 3, respectively). The Time × Stage, Time × Variety (Cultivar) and Time × Stage × Variety (Cultivar) interactions were not significant ($P > 0.05$; Table 4.5, rows 4, 6); however, unlike the Lods site, the Time × Cultivar × Stage interaction turned out to be significant.

Table 4.5: Repeated measures analysis for within-subjects main and interaction effects at the Normandin site.

S. No.	Source	Wilk's Lambda	F value	p-value
1.	Time	0.0147	233.35	<0.0001***
2.	Time × Cultivar	0.0451	74.01	<0.0001***
3.	Time × Stage	0.6921	1.56	0.2759^{ns}
4.	Time × Variety (Cultivar)	0.6564	0.82	0.5337^{ns}
5.	Time × Cultivar × Stage	0.4195	4.84	0.0478*
6.	Time×Stage×Variety (Cultivar)	0.6307	0.91	0.4865^{ns}

*** significant at 0.1% level, * significant at 5% level, ns: not significant

The results of univariate tests (data not shown) for checking significance of within-subjects effects also suggested similar findings.

Between-subjects main and interaction effects showed cultivar type, stage of herbicide application, along with their interaction (Table 4.6, rows 1, 2, 4, respectively), to have a weak, if significant, effect ($0.01 < P \leq 0.05$), indicating that cultivar and stage effects were not independent. The variety effect was barely non-significant ($P = 0.0502$), providing evidence of greater relative differences in herbicide concentration level between varieties 45H28 and 45H29 (Roundup Ready), and 5030 and 5040 (Liberty Link) than at the Lods site.

Table 4.6: Repeated measures of analysis between-subjects main and interaction effects at the Normandin site

S. No.	Source	DF	Mean Square	F-Value	p-value
1.	Cultivar	1	2043.82	10.77	0.0112**
2.	Stage	1	1950.81	10.28	0.0125**
3.	Variety (Cultivar)	2	844.46	4.45	0.0502 ^{ns}
4.	Cultivar × Stage	1	1078.01	5.68	0.0443**
5.	Stage × Variety (Cultivar)	2	22.03	0.12	0.8918 ^{ns}

** significant at 5% level, ns: not significant

4.5.2 Herbicide persistence in soil at the Normandin site

Soil samples collected from the Normandin site were analyzed for herbicide residues. Given that sampling time was significant (Table 4.5), and considering that a significant difference by cultivar type was to be expected, since each of two cultivar types received different herbicides, at different rates, a simple ANOVA analysis was applied to each sampling time (1, 7 and 20 days after herbicide application), with the lower soil depth (0.15-0.30 m) again excluded as being nearly all-zero. Notwithstanding this data being excluded from ANOVA, it is clear (Figures 4.11 and 4.13) that 1 and 7 days after applications, herbicide levels in the upper topsoil (0-0.15 m) were consistently higher (at least 3-fold) than those in the lower soil depth (0.15-0.30 m). A similar, albeit less pronounced, trend was apparent after 20 days.

The results of classical ANOVA for the three times (after 1, 7 and 20 days of herbicide application) are presented in Table 4.7. While no factor was significant for samples taken at 20 days after herbicide application, as expected, cultivar was significant at both 1 and 7 days after herbicide application. The analysis of variance for 1 and 7 days after herbicide application shows

cultivar (RR *vs.* LL) to show significant differences ($P < 0.0001$) in herbicide concentrations in the topsoil (0-0.15 m). Since the variety effect was non-significant in two cases and not highly significant ($0.02 < P \leq 0.05$) in the other (Table 4.5), the mean values of concentrations of varieties of RR and LL was used to graphically represent the persistence of the associated herbicides (Figures 4.11 and 4.13).

4.5.3 Glyphosate and glufosinate residues in soil at 2-3 leaf stage application

Mean glyphosate and glufosinate concentrations (µg kg^{-1}) in soil, over a 20-day period, for 0-0.15 m and 0.15-0.30 m soil depths are shown in Figures 4.11A and B, respectively. Similar to the Lods site, the highest concentrations of both the herbicides remained at the soil surface, with little found at the lower soil depth (0.15-0.30 m), even 20 days after herbicide application (Figures 4.11A and B). Roughly, 80% of the initial amount (concentration detected 1 day after herbicide application) was lost in the first 20 days, following 2-3 leaf stage herbicide application to the crop (Figure 4.11). However, disappearance of glyphosate residues in soil could represent the transformation of the parent molecule (glyphosate) into its main metabolite, AMPA (aminomethyl phosphonic acid). In our study, we could not quantify AMPA, given its overlap with a large peak at the same retention time as the derivatizing reagent FMOC-Cl. Both Veiga *et al*. (2001) and Sancho *et al*. (1996) mentioned the difficulty in estimating AMPA in their studies.

Table: 4.7 Analysis of variance for herbicide concentrations in the top 0.15 m of the soil at the Normandin site for sampling days 1, 7 and 20 days after application

Day after Herbicide Application				
Source	**DF**	**Mean Square**	**F value**	**P value**
Cultivar (RR and LL)	1	486.31	1.67	<0.0001***
Stage	1	849.57	2.92	0.1029^{ns}
Variety	2	619.98	2.13	0.5654^{ns}
Cultivar × Stage	1	111.35	0.38	0.269^{ns}
Stage ×Variety	2	24.27	0.08	0.3409^{ns}
7 days after Herbicide Application				
Cultivar (RR and LL)	1	8580.31	106.38	<0.0001***
Stage	1	1193.01	14.79	0.0049**
Variety	2	501.19	6.21	0.0235*
Cultivar × Stage	1	1303.21	16.16	0.0038**
Stage × Variety	2	104.3	1.29	0.3261^{ns}
20 days after Herbicide Application				
Cultivar (RR and LL)	1	59.69	0.54	0.4822^{ns}
Stage	1	164.19	1.49	0.2565^{ns}
Variety	2	9.43	0.09	0.9186^{ns}
Cultivar × Stage	1	104.37	0.95	0.3584^{ns}
Stage × Variety	2	7.97	0.07	0.9307^{ns}

***, **, * significant at 0.1%, 1%, and 5% level, respectively, ns: non-significant

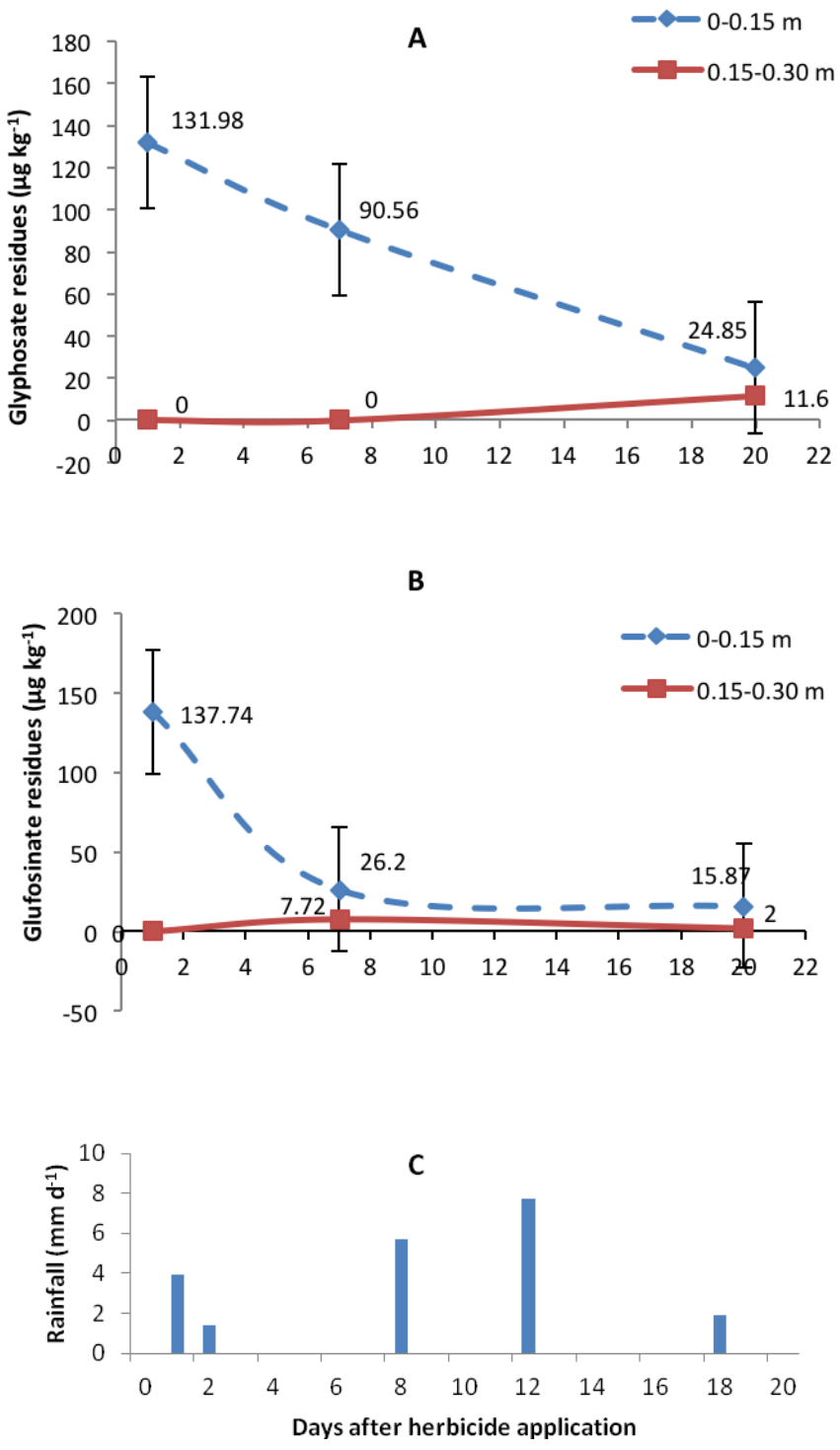

Figure 4.11: Residual levels of herbicides applied at 2-3 leaf stage in soil at the Normandin site at two depths (0-0.15 m and 0.15-0.30 m). A. Glyphosate, B. Glufosinate, C. Daily rainfall

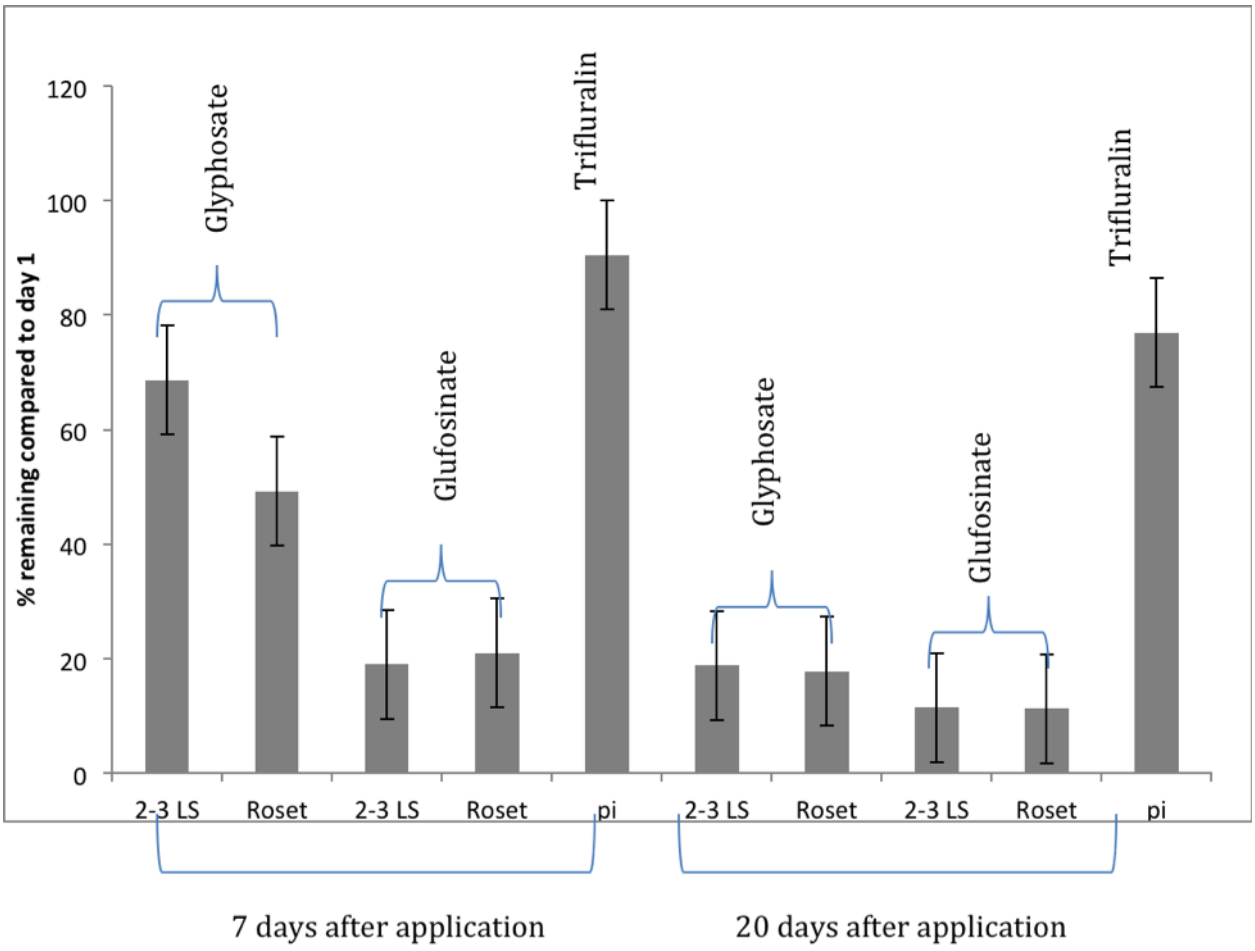

Figure 4.12: Percent herbicide residues, after 7 and 20 days of application, compared to herbicide residues present one day after herbicide application. Error bars show standard errors. In this figure, '2-3 LS' represents 2-3 leaf-stage, 'Roset' represents rosette and 'pi' stands for pre-incorporated stage of applications.

Roughly 9% of the initial (one day after application) glyphosate concentration was eventually detected in the lower soil layer. The total cumulative rainfall reported between the day of glyphosate application and the sampling day 20 days after herbicide application at the 2-3 leaf stage was 51.8 mm. Hence, rainfall is an important factor in promoting leaching or movement of

glyphosate to the lower depth. Further, soil texture can also influence the leaching potential of a chemical. The sandy loam texture of the Normandin soil could also explain greater leaching.

Similarly, in the case of glufosinate ammonium, maximum residual concentrations were also found at the top surface soil (0-0.15 m). At this depth, 81% and 88.47% of glufosinate ammonium was lost after 7 and 20 days of application (Figure 4.11). At the lower depth (0.15-0.30 m), 5.6% and 1.4% of the initial (Day 1 after application) glufosinate levels were found 7 and 20 days, respectively, after herbicide application. As the day of glyphosate and glufosinate application was the same, cumulative rainfall of 51.8 mm can be held, in part, responsible for glufosinate movement into the 0.15-0.30 m soil depth.

4.5.4 Glyphosate and glufosinate residues in the Normanidin site soil - rosette stage herbicide application

Glyphosate and glufosinate levels at the 0-0.15 m depth, subsequent to their application at the rosette stage, were lower than the levels detected following application at the 2-3 leaf stage (Figures 4.13 and 4.11, respectively). This can be attributed to the greater canopy interception of the herbicide spray by the larger canopy at the rosette *vs.* 2-3 leaf stage. As a result, a limited amount of these herbicides would have reached the soil surface. A similar trend was also seen at the Lods site.

After glyphosate application, its residual levels declined steadily from day 1 to 7 and then to day 20, whereas glufosinate residual levels declined more sharply from day 1 to 7 after its application (Figure 4.13). The lower rate of glyphosate decline indicated an increased persistence of glyphosate, as compared to glufosinate.

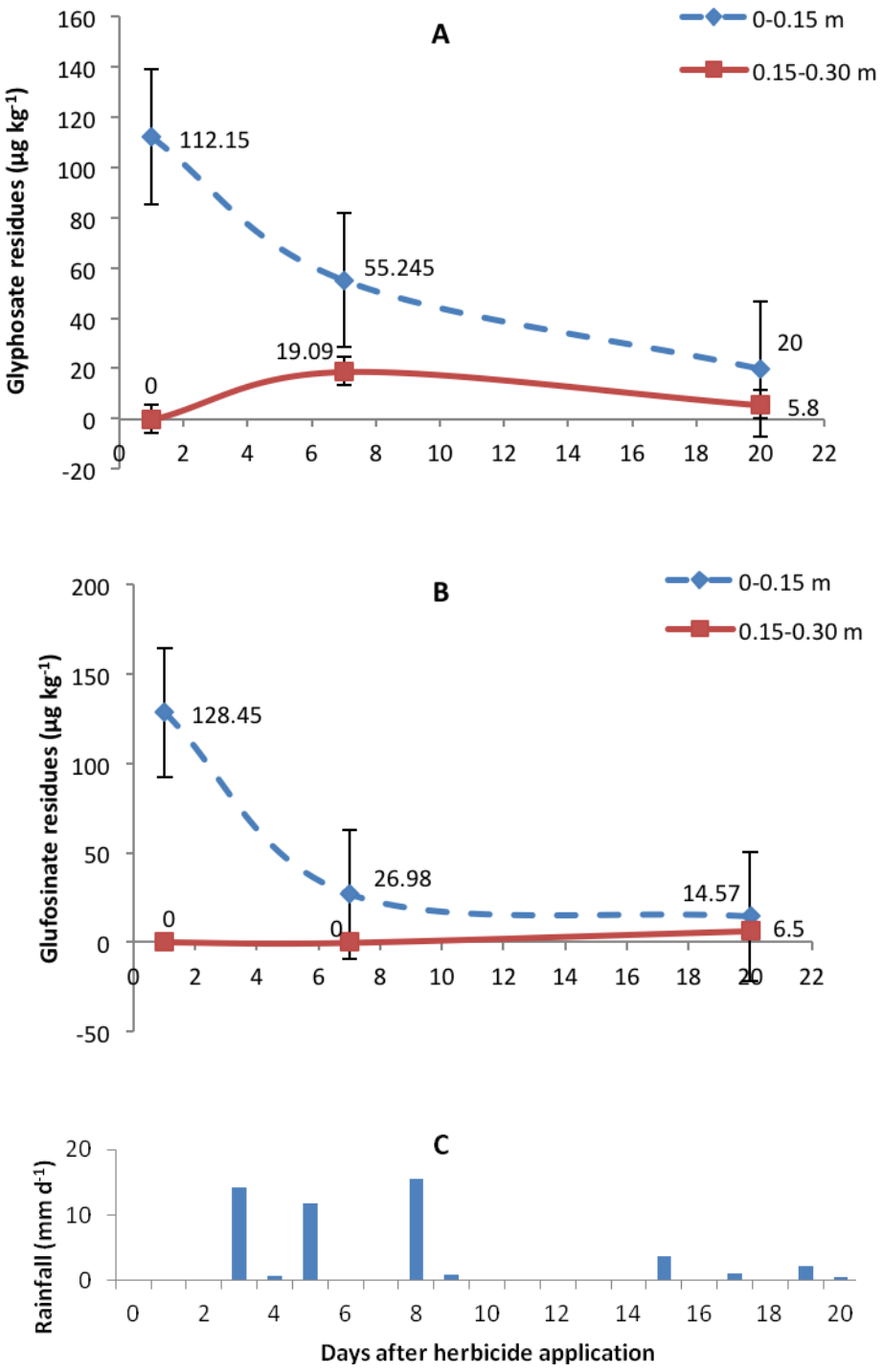

Figure 4.13: Residual levels of herbicides applied at the rosette stage in the Normandin site soil at two depths (0-0.15 m and 0.15-0.30 m). A. Glyphosate, B. Glufosinate, C. Daily rainfall.

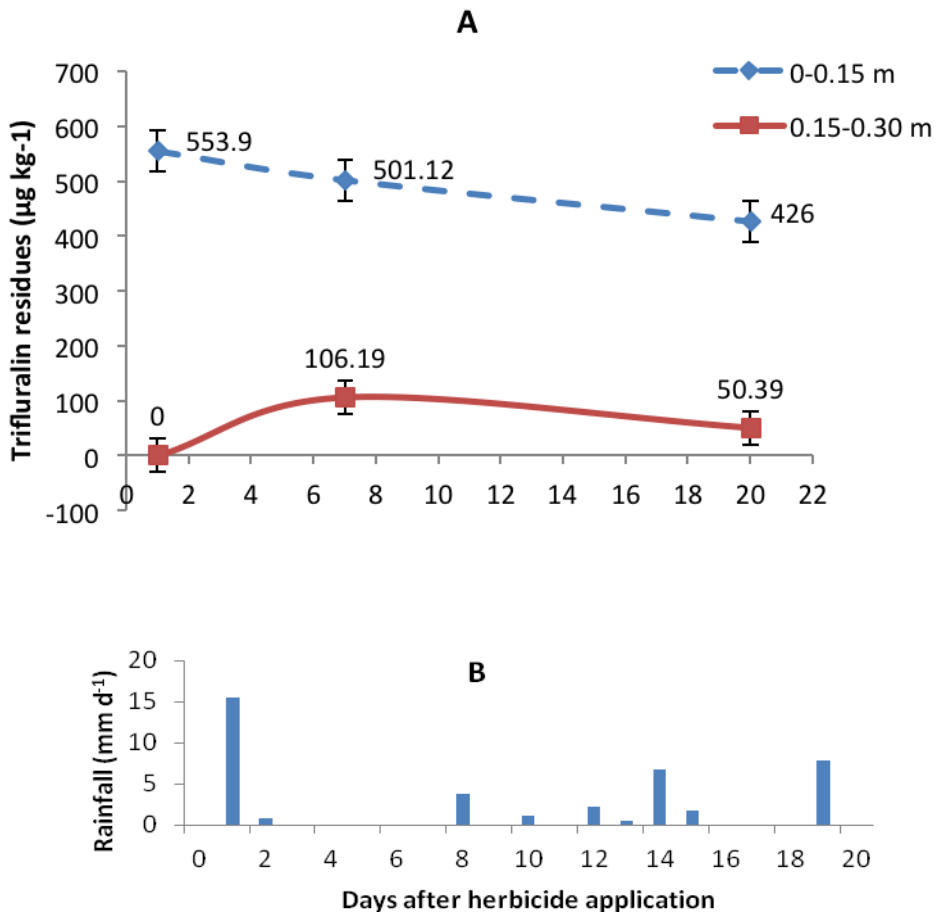

Figure 4.14 Residual levels of pre-emergence applied trifluralin in the Normandin site soil at two depths (0-0.15 m and 0.15-0.30 m). A.Trifluralin, B. Daily rainfall

However, at the lower depth (0.15-0.30 m), glyphosate was present after 7 and 20 days of herbicide application, with residual levels of 19.09 µg kg^{-1} and 5.8 µg kg^{-1} of soil, respectively. The day after (29 June 2012) glyphosate and glufosinate application, 15.5 mm of rainfall occurred (Appendix A-3) and the total cumulative rainfall during the 20 days after rosette stage herbicide application was 40.3 mm. The rainfall occurring the day after herbicide application could have contributed to the relatively higher glyphosate level (*vs.* the other application stage and *vs.* the Lods site) in the deeper soil layer. On the other hand, glufosinate ammonium was

only detected at this depth 20 days after its application (6.5 µg kg^{-1}). These lower concentrations at the 0.15-0.30 m soil depth indicate that, barring several heavy rainfall events, glyphosate and glufosinate ammonium would not leach down.

4.5.5 Residual concentrations of trifluralin in soils at Normandin site

At the 0-0.15 m soil depth, trifluralin levels at the Normandin site (Figure 4.14) were roughly the same as those found at the Lods experimental site (Figure 4.10) during the 20 days after the application of herbicide. The mean trifluralin concentration decreased gradually in the surface horizon of the sandy loam soil at the Normandin experimental location. After 7 days, the trifluralin concentration at the 0-0.15 cm depth had declined by 9.5%, compared to one day after application, while for 20 days after application, it had declined by 23% (Figure 4.11).

While 15.5 mm of rain fell on the day after trifluralin application, cumulative rainfall during the 20 days period after the trifluralin application was only 20.6 mm (Figure 4.14B). Hence, any decrease in concentration is likely to be tied to the Day 1 rainfall which would have led to good soil moisture content, capable of enhancing microbial degradation of trifluralin. Volatilization of trifluralin would not be expected because of low daily average temperature (14.2˚C) reported at the Normandin site during the period of study.

Trifluralin levels were also detected from the soil samples of 0.15-0.30 m depth at 7 and 20 days after application (Figure 4.14). Being a pre-incorporated herbicide, uneven distribution at application could also heighten the chances of getting soil sample with an unrepresentatively high or low amount of herbicide.

On the other hand in Figure 4.11, 49-68% of glyphosate and 19-21% of glufosinate remained 7 days after its application (compared to soil levels the day after application), while 20

days after application 17-18% and 11% remained, respectively. Comparatively, 90% of trifluralin remained after 7 days, and 77% remained after 20 days. Thus, the trend in herbicide dissipation rate was similar to those observed at the Lods site, i.e., trifluralin < glyphosate < glufosinate.

4.6 Half-lives of herbicides

The dissipation of glyphosate, glufosinate and trifluralin at both locations in the surface soils could be explained by first order kinetics. The dissipation half-lives and kinetic rate constants of these herbicides in soil are shown in Table 4.8. Dissipation graphs with regression equations are provided in Appendix A-1 and A-2, respectively. In this study, half-lives of these herbicides with respect to the stage of herbicide application (2-3 leaf *vs* rosette) were also calculated. Our results showed that trifluralin has the longest half-life at both locations, as compared to glyphosate or glufosinate ammonium.

Table: 4.8 The kinetic rate constants and half lives of herbicides in the soils of Lods and Normandin sites.

Stage of application	Herbicide name	Rate constant (k)		Half life (days) DT_{50}	
		Lods site	Normandin site	Lods site	Normandin site
2-3 LS	Glyphosate	0.083	0.09	8.35	7.7
Rosette	Glyphosate	0.049	0.089	14.14	7.78
2-3 LS	Glufosinate	—*	0.102	—	6.79
Rosette	Glufosinate	—	0.104	—	6.66
Pre incorporated	Trifluralin	0.012	0.014	57.76	49.51

* not calculable due to zero values for glufosinate residues 20 days after application at Lods site.

Mamy *et al*. (2005) also reported longer half-life for trifluralin than for glyphosate while studying the environmental fate of these herbicides in three French soils. The half-life of trifluralin was 57.8 and 49.5 days at the Lods and Normandin sites, respectively, and is consistent with the value of 54.7 days, reported by Kim and Feagley (2002). The half-lives of glufosinate ammonium were 6.79 and 6.66 days, according to whether the herbicide application occurred at the 2-3 leaf stage or the rosette stage, respectively. Half-life of glufosinate ammonium could not be calculated for the Lods experimental location due to the disappearance of the compound after 20 days of its application. However, the half-life of glufosinate ammonium could be calculated for the Normandin site soil, due to its greater persistence compared to the Lods site. Glyphosate's half-lives ranged from 7.70 to 14.14 days (Table 4.8), and were thus greater than those of glufosinate. Many other authors also reported a greater persistence (half-life) of glyphosate than glufosinate ammonium (Accinelli *et al*., 2004; Laitinen *et al*., 2006; Siimes *et al*., 2006; Gregoire *et al*., 2010). Glyphosate showed a slightly longer half-life when applied at rosette stage than at the 2-3 leaf stage, which might be attributable to the larger canopy cover of the canola crop, which results in lower soil surface temperatures, which, in turn, can lead to a reduction in microbial activities (Dullinger *et al*., 2003), ultimately resulting in longer persistence or half-life of glyphosate. Half-life of glufosinate ammonium at 2-3 leaf stage was 6.79 days, whereas at rosette stage, its half-life was 6.66 days for the Normandin soil. These half-lives are essentially the same.

CHAPTER 5

Summary and Conclusions

The main objective of this research was to carry out a field study on the fate and transport of glyphosate and glufosinate ammonium herbicides, actively used in Roundup Ready and Liberty Link HR crops, cultivars resistant to glyphosate and glufosinate ammonium respectively. We also studied trifluralin, a herbicide used in conventional canola cultivars to assess the benefits of growing HR canola over conventional canola in terms of persistence of herbicide residues in soil. The field experimentation was conducted at two locations in Quebec, at the Lods site on the Macdonald campus of McGill University, and at Normandin, 500 km away from the first location.

The main conclusion from this study is that most (> 90%) of the residual concentrations of glyphosate and glufosinate ammonium herbicides, used in HR canola system, and of trifluralin herbicide, used in conventional canola, remained in the top 0-0.15 m soil depth. The very high adsorption coefficient (K_d value) of glyphosate at Lods site of silt loam soil also indicates that glyphosate would not leach very much in the soil. Moreover, adsorption of glyphosate in soil also increases with high clay and high aluminum content. High adsorptive property of glyphosate could keep it in the top few millimeters of the surface soil. This would be a cause for concern as surface water bodies can get polluted when runoff occurs on agricultural land. Based on half-lives, glyphosate and glufosinate ammonium persist for a shorter period of time than the soil-incorporated trifluralin. The longer the persistence of the herbicides, the greater are the chances for the herbicides to cause environmental pollution. The overall order of persistence was trifluralin > glyphosate > glufosinate ammonium. It could be argued that all the three herbicides used in this study were strongly adsorbed to the soil particles, which makes them resistant to

runoff and/or leaching. But significant pollution could be expected from sediment transportation to water bodies during major rainfall/runoff events. Secondly, the rate of application for trifluralin, on an active ingredient per hectare basis, is almost double that of the HR canola herbicides. Hence, soil residues would be lower in case of herbicides used in transgenic canola as compared to trifluralin. Moreover, the present study also suggests that glyphosate and glufosinate ammonium, when applied at 2-3 leaf stage, leave greater residual concentrations of herbicides than when applied at the rosette stage.

Rainfall events, occurring soon after herbicide application, seem to play an important role in the leaching process of glyphosate and glufosinate ammonium. More leaching happened at the Normandin site than at the Lods site, confirming the role played by coarser-textured soils in the leaching process.

5.1 Recommendations for Future Research

Future research work could be done by using another commonly grown herbicide resistant canola cultivar, called "Clearfield", which is a non-transgenic variety developed through mutagenesis, and resistant to the herbicide Imazethapyr [5-ethyl-2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid]. Monitoring of the fate and persistence of glyphosate and trifluralin may be expanded to throughout the growing season of canola, and up to at least 100 days in Quebec soils because of their longer half-life. Soil sampling should be done for the top few millimeters also as this can confirm the higher herbicide concentrations and thus allow us to fully understand the herbicide pollution risks associated with soil erosion, surface runoff, etc.

CHAPTER 6

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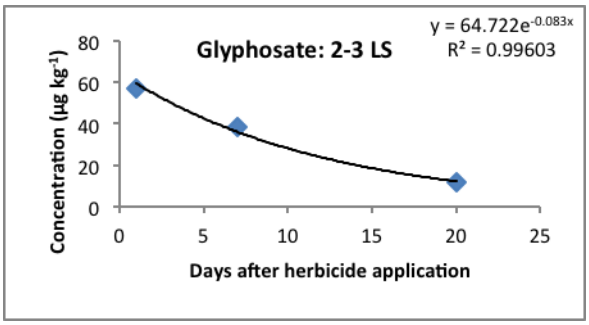
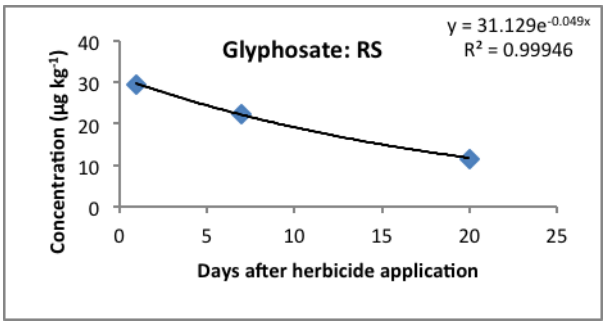
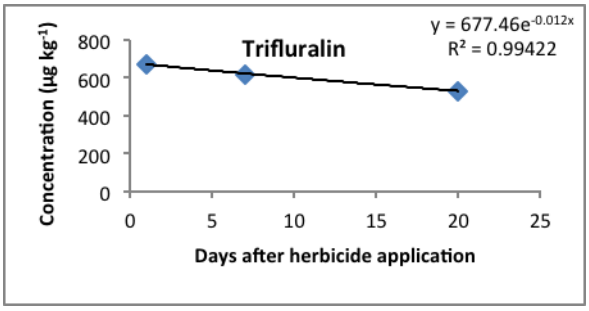
Appendix

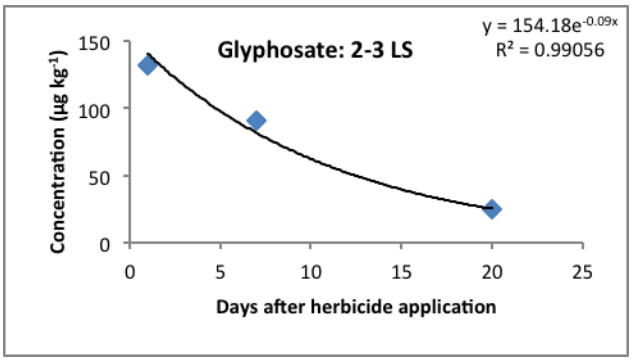
Appendix A-1 Regression equations and dissipation graphs of glyphosate and trifluralin at the Lods experimental site

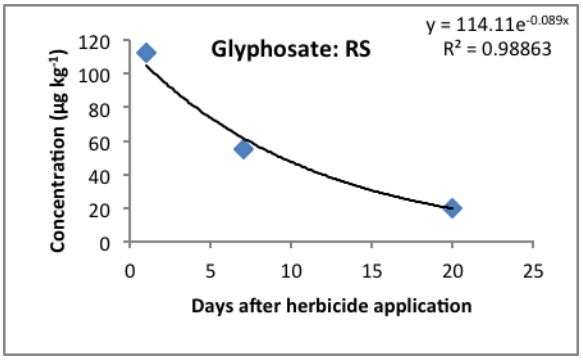


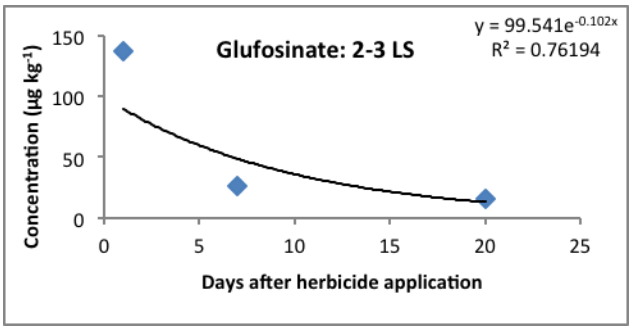


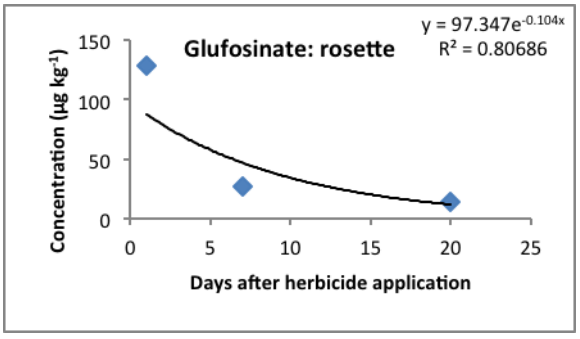


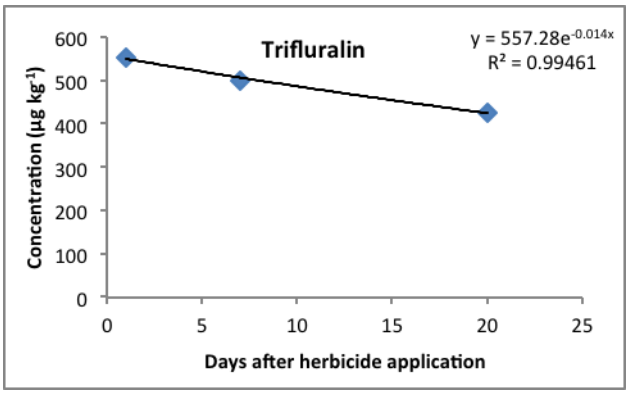
Appendix A-2 Regression equations and dissipation graphs of glyphosate, glufosinate and trifluralin at the Normandin experimental site.











Appendix A-3 Daily rainfall and temperature data in 2011 for the canola growing season at the Lods and Normandin sites.

Dates	Macdonald Campus		Normandin	
	Rainfall (mm)	Av. Temp.(˚C)	Rainfall (mm)	Av. Temp.(˚C)
1st June 2012	0	22.6	3.9	19.4
2nd June 2012	0	13.6	1.4	8.1
3rd June 2012	0	14.8	0	10.9
4th June 2012	0	14.9	0	9.4
5th June 2012	0	18.3	0	13.4
6th June 2012	0	19.3	0	13.0
7th June 2012	0	21.8	0	18.1
8th June 2012	1.4	25.8	5.7	15.8
9th June 2012	0.4	22.7	0	12.8
10th June 2012	0	18.5	0	11.4
11th June 2012	0.2	17.8	0	12.3
12th June 2012	16.6	16.8	7.7	12.1
13th June 2012	1.6	13.6	0	14.0
14th June 2012	0.2	17.0	0	16.5
15th June 2012	0	21.3	0	19.5
16th June 2012	0	23.2	0	17.9
17th June 2012	0	21.9	0	16.7
18th June 2012	0	20.1	1.9	14.6
19th June 2012	0	18.1	0	12.9
20th June 2012	0	18.0	0	15.2
21st June 2012	0	20.0	1.3	16.2
22nd June 2012	0	20.1	0	16.2
23rd June 2012	9.8	21.2	0	17.1
24th June 2012	11.2	17.8	14.2	14.6
25th June 2012	4	18.7	0.7	13.8
26th June 2012	0.2	19.9	11.8	16.7
27th June 2012	0	21.7	—	18.7
28th June 2012	7	22.2	0	18.9
29th June 2012	0.2	17.0	15.5	18.0
30th June 2012	0	18.0	0.8	18.6
1st July 2012	0.2	21.3	0	19.2
2nd July 2012	0	23.6	0	20.8
3rd July 2012	0	24.8	0	20.6

4th July 2012	0	24.6	0	18.7
5th July 2012	0	24.1	0	17.2
6th July 2012	3	21.5	3.7	17.0
7th July 2012	0	18.7	0	16.5
8th July 2012	6	19.6	1.1	16.1
9th July 2012	0.4	20.2	0	17.9
10th July 2012	0	22.5	2.2	16.8
11th July 2012	3	25.0	0.5	21.3
12th July 2012	0.2	24.5	6.8	18.0
13th July 2012	11.6	19.0	1.8	16.6
14th July 2012	0.2	19.5	0	18.6
15th July 2012	0	20.1	0	19.5
16th July 2012	0	23.3	0	19.4
17th July 2012	0.6	25.9	7.9	22.0
18th July 2012	0	24.2	0	16.7
19th July 2012	0	21.4	0	15.3
20th July 2012	0	23.9	8.7	17.4
21st July 2012	0.6	29.8	2.9	16.7
22nd July 2012	0.2	28.0	8	22.1
23rd July 2012	0	25.8	4.4	17.5
24th July 2012	0	20.2	0	17.3
25th July 2012	0	19.2	0	16.9
26th July 2012	1	21.4	17.9	15.5
27th July 2012	0	21.1	0	17.2
28th July 2012	0	22.0	2	17.8
29th July 2012	8.6	22.5	7.5	22.8
30th July 2012	0	21.3	0	16.0
31st July 2012	0	21.8	19.4	16.8
Total	**88.4 mm**		**159.7 mm**	