THE IMPACT OF CROP ROTATIONS ON THE FATE OF TRIFLURALIN AND GLYPHOSATE IN SOIL

BY

DENNIS MUC

A Thesis Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department Of Soil Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Muc, Dennis. M.Sc., The University of Manitoba, May, 2002. The Impact of a Potato Rotation on the Fate of Pesticides in Soil. Major Professor; Dr. Annemieke Farenhorst.

Agricultural pesticides are routinely applied to maximize crop production worldwide, with the largest percentage of pesticides belonging to the herbicide group. Since the pesticides that are not utilized by the target organism may contaminate the environment, it is essential to study their behavior in the soil environment. Pesticide sorption and mineralization are the two most important factors governing pesticide behavior in the soil.

This study examined the sorption and mineralization behavior of glyphosate and the persistence, sorption, volatility, and mineralization of trifluralin in soil from a potato rotation study conducted at the Manitoba Crop Diversification Centre (MCDC) located near Carberry MB. Experiments were also conducted to quantify the sorption and mineralization of glyphosate in soils amended with nitrogen and phosphorus fertilizers and humic acid. Standard batch equilibrium experiments were conducted to quantify the sorption of the herbicides while soil microcosms, were utilized to monitor herbicide mineralization and volatilization. Organic solvent extraction was used to determine the field persistence of trifluralin.

Sorption of both glyphosate and trifluralin approached 100%, in all experiments, with organic matter exerting a stronger influence for trifluralin sorption.

Mineralization of glyphosate proceeded at a much faster rate over 168 days than did trifluralin. Glyphosate mineralization in the soils amended with high amounts of nitrogen fertilizer and humic acid occurred at a significantly lower rate than the control soils and soils containing low amounts of fertilizer and humic acid amendments.

In most instances, the potato crop rotation did not significantly influence the sorption and mineralization of the herbicides in soil. The strong binding of glyphosate and trifluralin in soil would mitigate leaching of the herbicides through the soil profile, therefore groundwater sources below the experimental site would be unaffected by herbicide contamination. However, the strong sorption of the herbicides may cause it to be transported off-site by wind erosion.

FORWARD

The following thesis was prepared using the manuscript format outlined in the Guide to Thesis Preparation for Graduate Students in the Department of Soil Science. Chapter 9 will be submitted for publication to refereed journals. The manuscript will also include a co-author, Dr. Annemieke Farenhorst, who is also the major professor and advisor.

CHAPTER 1

Introduction

Pesticides are routinely used for the control of weeds, diseases, and insects in agricultural crops, with herbicide applications accounting for the single largest percentage of pesticides applied worldwide. After pesticides are applied to field crops, a portion may not be utilized by the target organism and therefore could pose a threat to the environment. Soil-incorporated, as well as foliar applied herbicides, may pose a hazard to non-target organisms, when present in surface or groundwater, or in the atmosphere. Therefore, it is essential to predict the behavior of herbicides in the soil environment in order to assess the risk they may pose environmentally. Pesticide sorption and mineralization are the two mechanisms largely influencing the behavior of pesticides in soil.

The soils for this study were obtained from the potato rotation study conducted at the Manitoba Crop Diversification Centre (MCDC) near Carberry MB. The study had been in progress for three years at the time of soil sampling. The overall objective of the rotation study was to define sustainable and viable potato rotations in Manitoba, as the industry continues to expand in this province. Within this larger research program, this study was undertaken to evaluate the fates of the herbicides trifluralin and glyphosate in the soil environment and complements the work conducted by the scientists at the MCDC research station.

Current agricultural practices rely heavily on pesticide input for weed, disease, and insect control. Glyphosate-tolerant crops are an economically attractive commodity utilized by farmers, as weeds are easily controlled with inexpensive herbicide products such as Roundup (glyphosate as the active herbicide ingredient) instead of using multiple herbicide products. Because glyphosate is now widely applied onto agricultural fields, it is important to understand the behavior of this herbicide in the soil environment.

Trifluralin is another herbicide commonly applied by farmers and is the active ingredient found in products such as Treflan and Rival 10G. Trifluralin is a soil-incorporated herbicide widely applied in production systems growing canola, an important crop in Western Canada. However, the persistence of this herbicide in soil beyond the season of application may cause crop injury problems where sensitive crops such as cereals are grown in rotation (Morrison et al., 1989). Understanding factors that influence trifluralin persistence in soil is important in defining management systems that reduce the risk of herbicide-carryover problems.

The soil mineral but more importantly, the soil organic matter (SOM) content, largely determines the sorption-desorption behavior of most pesticides in soil. Plant residues introduced into the soil are the main source of organic matter (Haider, 1992) and various crops grown are expected to have different organic matter inputs into the soil environment (Janzen, et al. 1992). The soil organic matter content in the soil, which in turn may influence the microbial population, also largely determines the mineralization rates of pesticides in the soil (Scow, 1993; Paul and Clarke, 1996).

The degradation of trifluralin and glyphosate in the soil environment is primarily accomplished through microbial activity and a number of bacterial and fungal species have been isolated that achieve this task (Messersmith et al., 1971; Franz et al., 1997). Soil microbial activity is influenced by soil nutrient content, particularly nitrogen and phosphorus, and soil organic matter content (Berger et al., 1996), which in turn may be affected by farm management practices such as tillage, crop grown and fertilizer practices (Kanal and Kolli, 1996).

There are a limited number of studies that examine the impact of long-term crop rotation cycles on pesticide behavior in soil, however increased pesticide degradation was noted as the cropping cycles were extended beyond the first year (Berger et al., 1996; and Piutti et al., 2002). I found no studies that addressed the research question whether the type of crop grown in a particular year could influence the behavior of a pesticide applied following harvest, or in the next cropping year. This study addresses whether short-term changes (three-year rotation) in the type of crop grown, could influence the behavior of pesticides in a Chernozemic silty clay loam soil belonging to the Wellwood series.

The soil organic matter fraction is largely responsible for the sorption of trifluralin in soil (Solbakken et al., 1982) while the soil mineral fraction exerts the greatest influence on glyphosate sorption in the soil (Sprankle et al., 1975). However, soil organic matter, in particular the humic fraction, can influence glyphosate sorption in soil (Piccolo et al., 1996), as does the phosphate content (de Jonge et al., 2001). Therefore, the second objective of this study was to determine whether the amount of nitrogen, phosphorous and humic acid (the most important soil organic matter fraction responsible for sorbing

most pesticides in soil) amendments in soil had an impact on the behavior of glyphosate in the soil.

The studies with trifluralin utilized in field and laboratory experiments. The first experiment focused on a field and laboratory studies to assess the amounts of trifluralin extracted from various rotation plots where the herbicide had been soil-incorporated the previous autumn. The second experiment was conducted in order to determine the sorption-desorption behavior of trifluralin from the rotation. The third experiment was conducted in the laboratory to determine the mineralization and volatilization rates of the herbicide in soil.

The second set of experiments was conducted utilizing laboratory experiments to determine the sorption-desorption and mineralization of glyphosate in soil from the potato crop rotations. Sorption and mineralization experiments were also designed to determine whether nitrogen and phosphate fertilizers and humic acid amendments had an influence on glyphosate behavior in soil.

CHAPTER 2

Literature Review

2.1. Fate of Pesticides in the Soil Environment

Agricultural pesticides are an indispensable component in modern agriculture as they are highly beneficial for controlling pests in field crops, but only if they remain in the target area of application. Soil-applied pesticides, as well as the portion of foliar-applied pesticides that enter the soil environment, are subjected to a number of chemical, physical and biological processes in the soil environment. The pesticide that is not utilized by the target organism may undergo photodegradation, chemical and microbial degradation, sorb onto soil constituents, or move from the target site of application by volatilization, leaching and erosion (Table 2.1). Any given pesticide will most likely undergo more than one of these processes and therefore the persistence and movement off-site of different pesticides will vary. Pesticides that move from the target site may contaminate groundwater, surface waters, other soil environments and the atmosphere. This is a serious environmental health concern as pesticides can have adverse effects on non-target organisms, including humans.

Table 2.1 Influence of pesticide characteristics on pesticide fate processes in soil.

	Importance to pesticide dissipation in soil
Sorption	
Mineral soil particles	Clay particles play an essential role in sorption of cationic and protonated weakly-basic pesticides. Main sorption mechanism of glyphosate.
Soil organic matter	Most important role in sorption of most pesticides. Main sorption mechanism for trifluralin.
Transformation	
Photodegradation	Least important transformation of pesticides but significant for trifluralin if not incorporated into soil.
Chemical degradation	Important for the triazine group but not very common for other pesticides.
Biodegradation	Most important transformation process for all organic pesticides.
Transport	
Volatilization	Important for pesticides with a high vapor pressure. Occurs with trifluralin if not properly incorporated into soil.
Leaching	Significant for pesticides with a low sorption potential.
Water erosion	Low sorption potential pesticides; transported in the water phase. High sorption potential pesticides: transported in the sediment phase.
Wind erosion	Occurs with pesticides with high sorption potential. Occurs with glyphosate and trifluralin.

A generalized theory regarding the behavior of pesticides in the soil environment is difficult as the soil system is heterogeneous, containing numerous microenvironments and different soil constituents, capable of influencing the behavior of a pesticide molecule contained within the soil matrix.

Organic pesticides can be classified into four different categories, depending on their molecular structure (Table 2.2). Most pesticides are non-ionic, while others may be classified as cationic and ionizable, and as weakly basic or weakly-acidic pesticides. Pesticide properties exert a large influence on the degree and nature of pesticide bonding on the soil constituents. For example, protonated (or weakly-basic) and cationic pesticides may strongly sorb onto clay minerals by cation exchange mechanisms (Koskinen and Harper, 1990). Non-ionic pesticide sorption may occur through the partitioning of the chemical between the aqueous phase and the hydrophobic organic matter in soil (Koskinen and Harper, 1990). The retention of the non-ionic pesticides occurs on the hydrophobic sites of humic substances, which are not conducive to sorption by polar water molecules (Senesi, 1992).

Table 2.2 Classifications of organic pesticides.

Non-ionic	Weakly basic	Weakly acidic	Cationic
Trifluralin	**Atrazine**	**Glyphosate**	**Paraquat**
Ethafluralin	**Metribuzin**	**MCPA**	**Diquat**
Triallate	**Simazine**	**2,4-D**	

2.1.1 Sorption Processes

Pesticide sorption by soil is the dominant mechanism influencing the fate of pesticides in the soil environment. For example, sorption strongly determines the rate of transport of pesticides to the atmosphere, groundwater and surface waters (Koskinen and Harper, 1990). Sorption refers to a general retention process that includes the following specific processes: 1) adsorption, which is the accumulation of a pesticide at either the soil-water or the soil-air interface, 2) absorption, which occurs when the pesticide directly enters into soil constituents such as clay minerals and soil organic matter, and 3) precipitation, in which the pesticide forms on soil surfaces or covalently bonds with the soil particle surface, making absorption into soil particles difficult (Koskinen and Harper, 1990).

The physical and chemical properties of the soil exert a strong influence on the behavior of pesticides in the soil (Koskinen and Harper, 1990). Soil organic matter primarily controls pesticide sorption processes and therefore affects degradation, transport, and the biological activity of organic chemicals in the environment (Senesi, 1992; Benoit et al., 1999). The mineral fraction, in particular the clay mineral content, will also play an important role in pesticide sorption behavior in soil. For example, soils high in montmorillonite clays, which have a high surface area than kaolinite clays, may be expected to exert greater sorption for pesticides (Sprankle et al., 1975). Pesticide sorption by clays is often linked to the cation-exchange capacity of the mineral and the amount surface hydroxyl groups that may participate in hydrogen bonding.

2.1.1.1 Sorption onto Inorganic Surfaces

The primary sorption mechanisms for pesticides to inorganic soil colloids are cation and anion exchange, protonation and ligand exchange (Koskinen and Harper, 1990). The inorganic solids in soil are composed of crystalline and noncrystalline, amorphous minerals, including primary (e.g., quartz and feldspars) and secondary minerals (e.g., phylosillicate clays, carbonates and oxides). Secondary minerals, due to their chemical structure, high surface area and chemical reactivity, exert a much stronger influence on soil sorption of pesticides relative to primary minerals (Koskinen and Harper, 1990). The inorganic hydroxyl groups are the most abundant and reactive functional groups for these minerals (Koskinen and Harper, 1990). Soil colloids such as clays and oxides often carry a negative or positive charge on the external or internal surfaces (Brady and Weil, 1999). The negative charge is primarily due to isomorphic substitution during mineral formation where a cation of lesser charge will substitute for one of similar size and a higher charge (eg. Mg^{2+} for Al^{3+}). The type of secondary mineral will determine the nature of the internal and external mineral surface, therefore influencing pesticide sorption. For example, the surface area of montmorillonite is approximately 800 m^2/g and kaolinite is 20 m^2/g (Brady and Weil, 1999), therefore montmorillonite is more reactive to cationic or ionizable weakly basic pesticides. The inorganic hydroxyl groups are the most abundant and reactive functional groups on clay minerals (Koskinen and Harper, 1990).

2.1.1.2 Sorption onto Organic Surfaces

The bonding mechanisms largely responsible for pesticide sorption onto organic surfaces are van der Waals forces, hydrogen bonding, cation and water bridging, anion exchange, cation exchange, ligand exchange and protonation (Koskinen and Harper, 1990). The organic component of the soil exerts an extremely important influence on the chemical and physical properties of the soil and is generally the most important component for the sorption of pesticides (Benoit et al., 1999; and Piccolo et al., 1996).

Soil organic matter can be conveniently classified into two main groups of compounds, nonhumic substances and humic substances (Stevenson, 1972). The former is represented by the unaltered remains of plant and animal tissues and includes classes of organic compounds such as carbohydrates, proteins, fats, waxes and resins. The latter group represents chemically and biologically modified substances that do not resemble their parent organic compounds, and can be further classified into humic and fulvic acids, and the humin fraction.

Humic substances (HS), the most reactive portion of organic matter in soils, can best be described as being highly acidic, yellow to black colored, high-molecular weight polyelectrolytes. Their ability to combine with organic molecules such as pesticides is primarily due to a high content of oxygen-containing functional groups, including COOH, phenolic-, aliphatic- and enolic-OH, and C=O structures of various types (Stevenson, 1972). Other functional groups present are the amino, heterocyclic amino, imino, and sulfhydryl groups, which are also capable of reacting with organic pesticides. The principal fractions of HS, humic acids (HA) and fulvic acids (FA), strongly interact

with pesticides due to their polydispersed nature and polyelectric character, their reactive surface properties, as well as the presence of various chemically-reactive functional groups, free radical moieties, and hydrophilic and hydrophobic sites in their molecular structures (Senesi, 1992).

It has been reported that pesticide sorption onto soil organic matter, in particular the humic fraction, may provide a temporary protection from microbial attack (Pignatello, 1989). However, microbial activities may increase pesticide degradation in soils with high nonhumified organic matter content and a high pesticide sorption coefficient (Benoit et al., 1999). Nonhumified organic matter may play a significant role because it is subject to active biological transformations. Some of the microorganisms involved in the degradation of fresh organic matter constituents can also degrade organic pesticides

Sorption processes of pesticides may vary from complete reversibility to total irreversibility as once sorbed on HS, a pesticide may be easily desorbed, desorbed at various levels, or not at all (Senesi, 1992). The extent of sorption depends on the amount of both the HS and the pesticide, and their properties, including the size, shape, configuration, molecular structure, chemical functions, solubility, polarity, polarizeability and charge distribution of interacting species, and the acid or basic character of the pesticide molecule (Senesi, 1992).

2.1.2 Pesticide Transformation Processes

Once the pesticide has entered the soil environment, transformation from the parent compound into one or more degradation products may occur. These transformation agents involve both biotic and abiotic mechanism. The main types of mechanisms are photodegradation, chemical degradation, and biotic or microbial degradation. The ultimate step in pesticide degradation is a process called mineralization, where microorganisms transform an organic compound into inorganic compounds, including CO_2 produced by respiration.

2.1.2.1 Photodegradation

Sunlight or photolysis is one of the processes affecting the loss of pesticides from the exposed soil environment (Wolfe et al., 1990). Photolysis is the process in which ultraviolet (UV) or visible light causes transformation of a compound and this process can chemically transform pesticides to substances with different toxicity and initiate the process towards mineralization (Wolfe et al., 1990). For a photoreaction to occur the compound has to absorb light energy, either directly or indirectly (Watkins, 1979). And although exceptions exist, most pesticides do not photolyze strongly otherwise their lifetimes would be insufficient for effective applications.

2.1.2.2 Biodegradation

The soil microbial population, whether they are bacteria, fungi or actinomycetes are perhaps the most important mechanism for transformation of pesticides in the soil environment (Benoit et al., 1999). Microorganisms exhibit two ecological strategies towards substrate assimilation or metabolism: mineralization and cometabolism (Felsot and Shelton, 1993). In the mineralization strategy, the absorbed substrate is broken down into smaller molecules, which are further metabolized via energy-generating pathways. In this case, the biomass of the population increases at the expense of the substrate. The principle metabolic end product is CO_2 along with other inorganic components. From an environmental point-of-view, the complete metabolism of an organic pesticide is desired for mitigating environmental contamination (Bollag and Liu, 1990). The presence of biodegradable pesticides in a natural ecosystem can cause proliferation of the active microbial flora and concurrently increase the rate of decomposition of the applied pesticide (Bollag and Liu, 1990).

During cometabolic microbial degradation, the organic pesticide does not serve as an energy, nutrient or carbon source for the microorganism (Bollag and Liu, 1990). Many pesticides are incidentally transformed in soil by this mechanism (Felsot and Shelton, 1993). Cometabolism is a fortuitous mechanism, in which enzymes involved in catalyzing the initial reaction are often lacking in substrate specificity (Bollag and Liu, 1990). Cometabolism generally does not result in an extensive degradation of a certain substrate, but it is possible that different microorganisms can transform a molecule by

sequential cometabolic attacks or that cometabolic products of one organism can be used by another as a growth substrate (Bollag and Liu, 1990). The essence of commensalism between populations occurs when the conversion of organic molecules by one population is used for substrate by others (Atlas and Bartha, 1993)

2.1.2.3 Chemical Degradation

Hydrolysis is a significant process in determining the fate of many pesticides in the soil environment (Wolfe et al., 1989). In general, hydrolysis refers to the cleavage of a bond of the pesticide and the formation of a new bond with the O atom of water (Wolfe et al., 1990). In abiotic hydrolysis reactions, the hydrolysis rates can be a function of chemical parameters such as H ion activity (pH), dissolved organic matter, and dissolved metal ions (Wolfe et al., 1990). It is convenient to classify hydrolysis reactions into three categories: acid-mediated, base-mediated, and neutral (or pH/independent) reactions (Wolfe et al., 1990). In the case of acid-mediated hydrolysis, an acid catalyzes the bond breaking-bond making process and because the proton is not consumed in the reaction, the process is referred to as acid-catalyzed hydrolysis. In the case of base-mediated hydrolysis, hydroxyl (OH-) behaves as a nucleophile and is consumed in the reaction and the pathway is often referred to as alkaline hydrolysis. In the third type of hydrolysis, the rate of reaction is independent of the acid-base concentration (pH independent) and is often referred to as neutral hydrolysis.

2.1.3 Pesticide Transport Processes

After the application of pesticides on field crops there exist the potential for the chemical to move off-site and contaminate the environment. There are a number of mechanisms that facilitate such movement from the application site: pesticide volatilization, pesticide leaching through the soil profile, and pesticide movement in surface water runoff and with wind eroded particles.

2.1.3.1 Volatilization

Volatilization of pesticides into the atmosphere is a major process that contributes to the loss of pesticides from the applied target area. Pesticide volatilization from plant and soil surfaces is affected by a variety of chemical and environmental factors (Prueger and Pfeiffer, 1994). For example, potential volatility of a pesticide is related to the vapor pressure of the active ingredient, while the actual rate of volatilization is dependent upon microclimatic parameters that can modify the effective vapor pressure of the pesticide (Prueger and Pfeiffer, 1994). Microclimatic parameters that can affect volatilization are temperature (of both air and soil), water vapor pressure of the air immediate to the soil-plant-air interface, soil water content, solar radiation, and wind speed.

The dispersal of pesticide residues into the atmosphere involves two distinct processes (Taylor and Spencer, 1990). The first is the evaporation of the pesticide molecule into the air from the residues present on the soil or plant surfaces. The second is the dispersion of the resulting vapor into the overlying atmosphere by diffusion and turbulent mixing. In physico-chemical terms, the first process represents a phase change from a liquid or solid

state into a vapor while the second is the same process that controls the transfer of water vapor and other gases between soil and plant surfaces and the atmosphere (Taylor and Spencer, 1990).

The highest rates of volatilization are found where pesticide residues are exposed to the atmosphere after direct application to moist soil or plant surface whereas volatilization is greatly reduced by incorporation of pesticides into the soil (Taylor and Spencer, 1990). This has implications from both an environmental and agronomic perspective. Movement of the pesticide from the target area may contaminate the atmosphere and water sources as well as affect sensitive non-target organisms. Volatilization from an agricultural field will reduce the efficacy of the pesticide and may cause the need for repeated applications.

2.1.3.2 Leaching

Leaching of pesticides through the soil profile is another major pathway by which pesticides may be lost from the target area of application and there are a number of factors that contribute to pesticide leaching (Flury, 1996). For example, surface preparation or tillage practices may impact pesticide leaching. Conventional tillage operations have been reported to reduce soil organic matter, and hence aggregate stability, aggregate size, pore continuity and infiltration rate (Arshad et al., 1990). Under long-term zero tillage, increased infiltration results from reduced soil disturbance and development of stable and continuous pores (Elliot and Efetha, 1999). Conservation tillage may leave preferential flow pathways such as earthworm burrows and root

channels intact and therefore lead to preferential flow of pesticides into the lower soil profile (Flury, 1996).

Soil structure and texture may also contribute towards pesticide leaching. Coarse textured soils permit rapid infiltration of water and dissolved pesticides compared to fine textured clay soils. And fine-textured soils with large water-stable aggregates (granular structure) have greater water infiltration rates than massive (structureless) soils (Miller and Donahue, 1990).

Pesticide leaching is also related to the time of application and rainfall events (Flury, 1996). That is, the longer the time between pesticide application and a major rainfall event, the less pesticide leaching will occur. This is related to an increasing amount of pesticide being degraded, and a progressively greater sorption of pesticide residues onto the soil constituents.

2.1.3.3 Water Erosion

Pesticides are often lost from the applied target-areas via surface runoff. Specifically, pesticide runoff includes dissolved, suspended particulate, and sediment-adsorbed pesticides, which are transported by water from a pesticide-treated land surface (Leonard, 1990). Conditions most favorable for runoff are when pesticides are applied to foliage or crop residues immediately before an intense rainstorm on a soil that is nearly saturated from a previous rain (Leonard, 1990). Pesticide runoff has been shown to be highly seasonable and variable within years (Rawn et al., 1999). For example, pesticide runoff from fields is exacerbated in the spring by snowmelt and runoff and greater pesticide

transport has also been detected shortly after application times of the pesticide (Rawn et al., 1999).

Soil tillage after pesticide application may incorporate pesticides to lower depths and reduce the concentration at the immediate soil surface (Leonard, 1990). This reduces the amount of pesticides available for surface runoff.

2.1.3.4 Wind Erosion

In the absence of conservation practices, wind erosion remains one of the major forms of soil degradation on the Canadian prairies (Wall et al., 1995). Pesticides that are sorbed onto the soil particles may be therefore transported great distances and potentially contaminate surface waters as well as land areas where crops are grown and people live (Hawthorne et al., 1996; Larney et al., 1999).

2.1.4 Environmental Concerns

The widespread use of pesticides worldwide has led to increasing concern about their impact on the natural environment. Pesticide movement from target areas has the potential to contaminate soil, groundwater, surface water and the atmosphere, and adversely affect susceptible non-target organisms. Ingestion of pesticides has been associated with health problems that include cancer, nervous system disorders, birth defects, and male sterility (Goodrich et al., 1991).

Herbicides such as 2,4-D and bromoxynil have commonly been detected in surface waters and groundwater in western Canada (Waite et al., 1992; Rawn et al., 1999). Although the detected amounts did not exceed the guidelines of 4000 and 5000 ng/l,

respectively for 2,4-D and bromoxynil, set by the Canadian government, their presence has illustrated the fact that pesticides move from the target area.

A study conducted by Goodrich et al. (1991) showed that 74 pesticides were detected in groundwater sources in 38 states in the U.S. Pesticides such as atrazine and metolachlor, which are moderately sorbed in soil and relatively soluble in water, have the potential to move out of the root zone and contaminate groundwater. Once these contaminants reach groundwater, they can persist for many years causing potential health concerns for those using groundwater as their drinking source.

2.1.5 Agronomic Concerns

The risk of crop injury, in particular from herbicides, is a concern agricultural producers may face during the course of the growing season. For example, sulfonylurea herbicides are widely used in Canada for the control of annual broadleaf weeds in cereals and certain broadleaved crops. Canola and sunflower are sensitive to low doses of this group of herbicides, leading to crop injuries when the soil-residual herbicides persist in agricultural fields (Wall, 1997). Herbicides applied as sprays often miss the target plant or are washed off by rain and irrigation, leading to the deposition of herbicides on the soil surface. Subsequent soil disturbance by winds or other means may cause soil-sorbed herbicides to become airborne and then deposited downwind on plant foliage or soil, causing crop injury (Al-Khatib et al., 1992). Mark et al. (1989) found that imazaquin, imazethapyr and clomazone, which are used for weed control in soybean, can persist in the soil and cause injury to susceptible crops such as wheat and corn grown in rotation. This appeared more likely to occur in soil with a high adsorptive capacity for these

herbicides, rendering greater persistence of the pesticides in agricultural fields. Trifluralin, which is commonly applied as a pre-plant soil incorporated herbicide, may cause injury to cereal crops if the seeds are planted into soil containing trifluralin residues (Gerwing and Mckercher, 1992). Flax has also been found to be susceptible to trifluralin residues in soil (Nawolsky et al., 1992).

Pesticides can enter the atmosphere via application drift, evaporation, sublimation or erosion of treated soil. Once in the air, pesticides can be transported and returned to the earths surface via wet and dry deposition and potentially injure sensitive crops (Hill et al., 2002). Hill et al, (2002) conducted an indoor bioassay study using simulated rainfall containing the same concentration of dissolved phenoxy herbicides as was measured in natural rainfall in parallel studies and found that injury to beans and tomatoes in the field may occur.

2.1.6 Crop Rotations and Soil Organic Matter

A number of studies have quantified the soil organic input by various field crops (Janzen et al., 1992; Anonymous, 1993; Campell and Zetner, 1993; Kanal and Kolli, 1996; Angers et al., 1999). The production of soil organic matter has important consequences for pesticide behavior in soil as soil organic carbon content has been considered the single best predictor of pesticide sorption in soil (Oliveira et al., 1999).

Some of the crops grown in the potato crop rotations that are utilized at the MCDC research station can be expected to contribute different amounts of organic matter to the soil (Anonymous, 1993; Angers et al., 1999). A potato rotation study conducted in Atlantic Canada demonstrated that potatoes contributed very small quantities of organic

matter to the soil relative to cereals and forages (Anonymous, 1993). Intensive soil disturbance in potato production contributes to the destabilization of soil macroaggregates, resulting in the acceleration of soil organic matter decomposition through oxidation. Angers et al. (1999) found that a 10 yr potato cropping sequence that contained cereals and forage legumes had more than double the carbon input back into the soil than the continuous potato rotation. The greater amounts of crop residues, in particular the root matter, as well as less tillage events for the forages and cereals in the rotation subsequently account for the greater input of soil organic matter.

The amounts of soil organic matter input into the soil are also regulated by fertilizer practices, tillage, climate, soil texture, and topography (Janzen et al., 1992; Elliot and Efetha, 1999). The decomposition of soil organic matter is determined by the soil properties such as texture, calcareousness, biological activities and temperature and moisture conditions (Kanal and Kolli, 1996). Therefore, it is difficult to make generalizations regarding the amounts of soil organic matter inputs and stability by type of crop grown.

The formation of humic substances through the decomposition of plant residues is a complex biochemical process that is not well understood (Stevenson, 1994). However, once formed, depending on the environment, the half-life of the humic substances may range from 10 yr to centuries (Brady and Wiel, 1996). As the amount of crop residue input into the soil increases, the amount of relatively stable soil organic matter (i.e. humic substances) builds up over time. This may have a significant effect on pesticide behavior in soil.

2.1.6.1 Crop Rotation at MCDC

The crop rotation study at the MCDC site was conducted with the objective of defining viable potato rotations that ensure sustainable land management during the expansion of the potato industry in Manitoba. The research program is unique in that it examines six different potato crop rotations located at one site and covers almost every aspect of crop management. This is the largest potato rotation study being conducted in Canada at this time. Cereals (wheat and oats), canola and a forage legume (alfalfa) have been included in the rotations. The rotations range from two to four years in length. A wide variety of herbicides, insecticides, and fungicides are being applied to maximize crop production. Trifluralin and glyphosate are two herbicides that are commonly applied and thus have been chosen for this study.

2.2 Trifluralin

Trifluralin (2,6-dinitro-N,N-dipropyl-4-trifluomethylaniline) applied alone, or in combination with other herbicides, is extensively used in western Canada as a pre-emergence soil-incorporated treatment for the control of a variety of grassy and broadleaf weeds in cereal and oilseed crops (Smith and Aubin, 1994). The common formulations of the herbicide are as granular and emulsifiable concentrates. Typical application rates in Manitoba range from 1.1 kg/ha to 3.4 kg/ha depending on the soil texture and amount of organic matter in the soil.

Trifluralin belongs to the group of dinitroaniline herbicides and the chemical structure is shown in Fig. 2.2. while the chemical and physical properties are outlined in Table 2.2.

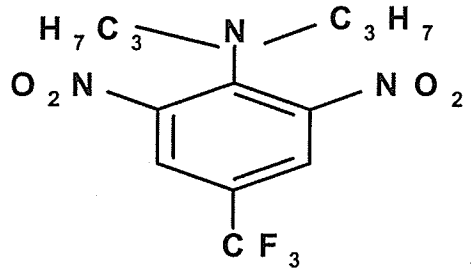

Figure 2.1 The trifluralin molecule.

Table 2.2 The chemical and physical properties of trifluralin (The Pesticide Manual, 1983; Reviews of Environmental Contamination and Toxicology, 1992).

Molecular weight	Solubility mg/L	Half-life (Days)	Soil sorption (Kd)	Vapor pressure	Toxicity LD_{50}
335.5	< 1 mg	60	1200 - 9850	1.1×10^{-4}	10,000

2.2.1 Trifluralin in the Soil Environment

After trifluralin is incorporated into the soil there are a number of pathways that the herbicide may take to dissipate from the soil environment. A portion is taken up by growing plants while the remainder may volatilize, degrade either chemically or biologically, or may move off-site via leaching and erosional processes. Losses from treated soils occur mainly by volatilization and biodegradation (Smith and Aubin, 1994). Research has shown trifluralin to be strongly sorbed to soils and due to this property and its low water solubility, leaching of this herbicide was found to be negligible (Peters and Weber, 1985).

2.2.1.1 Factors Affection Trifluralin Sorption

Studies have shown that trifluralin is strongly sorbed onto soil particles and the primary factor affecting sorption is the soil organic matter content (Bardsly et al., 1967; Peters and Weber, 1985; Pederson et al., 1995 and Solbakken et al., 1982). Trifluralin sorption also occurs, to a lesser degree, on the inorganic mineral fraction in the soil (Lafleur et al., 1978). Trifluralin's shape and molecular size and its behavior as a non-ionic compound facilitate binding to organic matter by van der Waals forces (Solbakken et al., 1982). The van der Waals forces are weak short-range bonds but the interactions are additive between trifluralin and high molecular weight molecules such as humic acid and thus lead to the overall production of strong, short-range bonds (Koskinen and Harper, 1990). Trifluralin has a very low solubility in water and is nonploar, therefore would not likely form H-bonds with the soil constituents.

2.2.1.2 Factors Affecting Trifluralin Mobility

A number of laboratory studies have indicated a very low leaching potential of trifluralin (Kim and Feagle, 1998; Malterre et el., 1998 and Malterre et al., 1997). This has been attribute to the low water solubility of trifluralin and its strong sorption on soil particles. Therefore, the potential for groundwater contamination of the herbicide is very negligible.

Trifluralin sorbed to soil particles may move off-site via wind and water erosion (Brown et al., 1995; Larney et al., 1999; Gouy et al., 1999). A study by Gouy et al. (1999) utilized simulated rainfall on experimental plots to generate a large load of eroded sediment particles. Very low concentrations of trifluralin were detected in runoff waters,

however, 90 % of the trifluralin detected was sorbed onto eroded and transported soil particles. The maximum amounts of trifluralin concentrations detected by Brown et al. (1995) in surface drainage water from a field experiment (that received 275 and 456 mm of rainfall during the two growing seasons and had applications of 480 g litre^{-1} of trifluralin) was 0.8 ug litre^{-1}, suggesting that the herbicide is relatively immobile in the soil environment.

Trifluralin has been detected in wind-eroded sediments and represents one mechanism for off-site movement of the herbicide (Larney et al., 1999). Larney et al. (1999), found trifluralin concentrations ranging from 312-to 353 ug kg-1 at heights of 10-100 cm in wind erosion samplers, which accounted for 1.4% of the herbicide applied. The soil-incorporation of the herbicide was deemed responsible for the low amounts detected.

2.2.1.3 Factors Affecting Trifluralin Volatilization

Trifluralin has the capacity to volatilize from the soil environment after application (Bardsley et al., 1967; Harvey, 1974; Spencer and Cliath, 1974; Savage and Barrentine, 1969). Volatilization of trifluralin is related to soil water content (Harvey, 1974; Spencer and Cliath, 1974). Volatilization rates were much higher in moist than dry soils. Soil organic matter content also influences trifluralin volatilization from soil (Bardsley et al., 1967). Volatilization losses decreased as the organic matter increased, supposedly due to the greater soil sorption of the herbicide. Soil temperature also has an impact on trifluralin volatilization. Savage and Barrington (1969) found that significantly more trifluralin was lost at 40°C than at 30°C. In addition, the depth of soil incorporation influences the amount of trifluralin volatility from soil (Savage and Barrentine, 1969;

Spencer and Cliath, 1974).The greatest amount of volatilization occurred from surface applications of the herbicide and the lowest when incorporation was at the 7.5 cm depth (Savage and Barrentine, 1969).

2.2.1.4 Factors Affecting Trifluralin Degradation

A number of studies have reported the biodegradation of trifluralin under both field and laboratory conditions (Messersmith et al., 1971; Parr and Smith, 1973; Probst et al., 1975; Carter and Camper, 1975; Wheeler at al., 1979; Golab et al., 1979). A study by Parr and Smith (1973), provided convincing evidence that microbial activity is the major process by which trifluralin degrades in the soil environment. Their evidence includes: a) enhanced degradation in the presence of an organic substrate, b) lack of trifluralin degradation in moist anaerobic environments after autoclaving, c) resumption of respiratory activity in the autoclaved system after reinoculation, which corresponded with increased degradative activity, and d) a temporary lag period or suppression of CO_2 respiration and trifluralin degradation from the presence of KN_3, a biological inhibitor, with simultaneous resumption of respiration and degradation soon after chemical dissipation of KN_3. Trifluralin appears to degrade in soils via oxidative (aerobic) and reductive (anaerobic) pathways (Helling, 1976). Studies have shown that trifluralin is degraded faster in anaerobic than aerobic soils (Parr and Smith, 1973). Oxidative pathways involve a series of oxidative dealkylation steps of the propyl groups, while reductive pathways show a reduction of the nitro groups. Other degradation mechanisms include: hydrolysis, cyclization and condensation, and a combination of all of these reactions resulted in the formation of more than 30 degradation products in soil (Golab et

al., 1979). For example, the metabolites detected by Wheeler et al. (1979) in an aerobic soil environment showed that trifluralin underwent dealkylation to monodealkylated and subsequently to a didealkylated product. Two benzimidazoles, (2-ethyl-7-nitro-1-propyl -5-(trifluoromethyl)benzimidazole) and (2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole) were also identified. The ultimate degradation of trifluralin produces CO_2 and water (Parr and Smith, 1973).

Soil microorganisms that degrade trifluralin have been isolated. Carter and Camper (1975), in a pure culture study, showed that the degradation of trifluralin by a soil fungus, *Paecilomyces* sp., resulted in several degradation products, two of which were identified as the corresponding benzimidazole of trifluralin and its monodealkylated derivative. In another experiment, utilizing soil enrichment cultures with trifluralin, Carter and Camper (1975) isolated eight species of bacteria from the genus *Pseudomonas* that appeared to have a mechanism that can metabolize or degrade the herbicide. Also the fungus *Phycomycetes mycotypha* was isolated and identified using agar plates in which trifluralin was the only known source of carbon and nitrogen (Messersmith et al., 1971).

Field and laboratory studies have shown that trifluralin is moderately persistent in soil (Helling, 1976; Wheeler et al., 1979; Gerwing and McKercher, 1992; Smith and Aubin, 1994; Corbin et al., 1994; Berger et al., 1996). Breakdown of ^{14}C-trifluralin to CO_2 by soil organisms in a laboratory incubation experiment accounted for only 3 and 5% of total loss in a sandy loam and silty clay soil, respectively (Messersmith et al., 1971). Wheeler et al. (1979) obtained a similar result in their laboratory experiments as, after 83 days, trifluralin losses through conversion to $^{14}CO_2$ ranged from 2.5 to 3.1% in a silty clay loam soil and from 1.4 to 2.0% in a sandy loam soil. A field study by Gerwing and

McKercher (1992) detected trifluralin residues in the field one year after application. Another field study by Corbin et al. (1994) found low levels of trifluralin present (0.06 – 0.14 kg/ha) at 30 months after the last trifluralin application. These figures translate into the half-life of trifluralin ranging from 8.7 to 14.9 months. Field persistence of trifluralin, however, is dependent upon many factors such as edaphatic considerations, rainfall, herbicide application dates and rates, method of application, and methods and depth of soil incorporation in the field (Smith and Aubin, 1994).

2.2.1.5 Factors Affecting Trifluralin Photodecomposition

Studies have shown that trifluralin is subject to photodecomposition, therefore soil incorporation of the herbicide is necessary soon after field application (Wright and Warren, 1965; Messersmith et al., 1971; Parr and Smith, 1973; Leitis and Crosby, 1974). Wright and Warren (1965) showed that when trifluralin was exposed to sunlight on a soil surface for a period of two hours, the herbicidal activity was significantly lower than that of the unexposed material. Letis and Crosby (1974) concluded that photodecomposition of trifluralin involves oxidative *N*-dealkylation, nitro reduction and cyclization, while dealkylation may be a free-radical oxidation by atmospheric oxygen.

2.2.2 Agronomic Concerns of Trifluralin

The field persistence of trifluralin beyond the season of application has been well documented by a number of studies (Gerwing and McKercher, 1992; Corbin et al., 1994; Smith and Aubin, 1994). Corbin et al. (1994) extracted detectable amounts of trifluralin residues 30 months after application. Consequently, the persistence of trifluralin residues

in the soil may result in crop injury to other rotational cereal crops during the following growing season (Morrison et al., 1989; Hartzler et al., 1989; Morrison et al., 1991; Nawolsky et al., 1992; Gerwing and McKercher, 1992; Corbin et al., 1994). Corn is sensitive to higher than recommended field rates of application and damage to wheat can occur if the crop is seeded into land that has been treated during the previous 21 months with trifluralin products and has received abnormally low amounts of precipitation (Anonymous, 1999). Injury to wheat is generally more severe when the conditions do not favor rapid emergence (e.g. low soil temperatures) and when the crop is seeded too deeply (Anonymous, 1999). Morrison et al. (1989) observed trifluralin injury symptoms at a very early stage of wheat crop development, with the greatest amount of injury occurring in the plots treated with the largest trifluralin applications. However, under favorable growing conditions, the yield loss would be minimal. Trifluralin injury may also occur in flax if the chemical is not uniformly distributed in the soil, if the flax is seeded deeper than 3 cm, or if seeding occurs while the seedbed is still cold (Nawolsky et al., 1992).

The phytotoxicity of trifluralin to field crops may be reduced as the organic matter of the soil increases (Helling, 1976; Solbaken et al., 1982). The strong binding of the herbicide to the soil organic matter will restrict the availability and therefore mitigate injury to field crops grown in soils which received previous applications of trifluralin.

2.3 Glyphosate

Glyphosate [N- (phosphonomethyl) glycine] is a nonselective broad spectrum herbicide extensively used in agriculture for the control of many annual and perennial

weeds (Piccolo et al., 1996). The common formulations include glyphosate present as the isopropyl amine salt and formulated as a solution, or glyphosate present as the monoammonium salt and formulated as a dispensable granule (Anonymous, 1999). Application rates vary according to type of weed targeted for control but range from 0.74 to 6.9 l/ha of active ingredient. The extensive use has led to numerous studies to determine glyphosate persistence and mobility in the soil environment.

The glyphosate molecule has a zwitterions structure containing three functional groups; the carboxalate, phosphonate and amine groups (Figure 2.1) (The Pesticide Manual, 1983). The carboxylate and phosphonate groups ionize at high pH and carry a negative charge. The chemical and physical properties of the herbicide are listed in table 2.2.

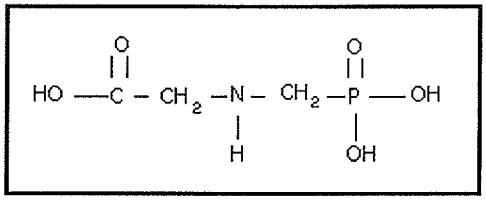

Figure 2.2 The glyphosate molecule.

Table 2.3 The chemical and physical properties of glyphosate (The Pesticide Manual, 1983; Reviews of Environmental Contamination and Toxicology, 1992).

Molecular weight	Solubility mg/L	Half-life (Days)	Soil sorption (Kd)	Vapor pressure	Acid pKa	Toxicity LD_{50}
169.1	1200	47	500 - 60000	0	2.3,5.86,10.9	5600

2.3.1 Glyphosate in the Soil Environment

After glyphosate enters the soil environment, it is subjected to sorption, transformation and transport processes. Glyphosate persistence in soil is therefore influenced by factors such as its chemical structure and physical properties, climate, soil properties, microorganisms present in the soil, and the agronomic cultural practices (Franz et al., 1997). Glyphosate half-lives in soil ranged from 18 days to 22.8 years (Sprankle et al., 1975; Rueppel et al., 1977; Nomura and Hilton, 1977), with a generally reported half-life of 47 days (The Pesticide Manual, 1983).

2.3.1.1 Factors Affecting Glyphosate Sorption

A salient characteristic of glyphosate is that herbicide activity through the soil is low, as a result of its sorption to the soil constituents (Grossard and Atkinson, 1985). Studies on soil sorption of glyphosate have proposed that a number of chemical and physical properties of the herbicide and the nature of soil constituents account for this strong sorption behavior. The earliest research by Sprankle et al. (1975) showed that glyphosate adsorption to the soil occurred rapidly within the first hour and continued slowly thereafter. They also discovered that soil type affects the adsorption of glyphosate as a sandy loam soil adsorbed less of the herbicide than a clay loam soil. When Sprankle et al. (1975) examined a cation-saturated bentonite clay, they found that glyphosate adsorption onto the clays decreases in the order of: Al+++> Fe+++> Zn++> Mg++> Ca++> Na+ – saturated clays. In addition, the study indicated that glyphosate is bound to the soil through the phosphonic acid moiety. A similar study by Glass (1987) also showed that

glyphosate sorption is related to the clay content of the soil as montmorillonite had a higher adsorptive capacity than either illite or kaolinite. This suggests that glyphosate could adsorb within the interlayer spaces of the clay minerals, and that glyphosate sorption appears to be related to the cation-exchange capacities of soils. When Glass (1987) saturated clay minerals with various cations, the adsorption order was the same as that found by Sprankle et al. (1975), and proposed that the ability of the cations on the clay surfaces to form coordination complexes was responsible for the adsorption of glyphosate by the cation-saturated clays.

The soil organic matter content, specifically the amount and type of humic substances, may also influence glyphosate sorption in soil (Nomura and Hilton, 1977; Piccolo and Celano, 1994; Piccolo et al., 1996). The proposed mechanism is hydrogen bonding as the glyphosate molecule contains various electronegative atoms, which can act as both hydrogen donor and hydrogen acceptor groups. Similarly, humic matter contains a large content of oxygen-containing functional groups, which have a large capacity for hydrogen bonding. A closer relation to the sorbing capacity of humic substances with glyphosate was revealed by their content of aliphatic C and their molecular size (Piccolo et al., 1996). In fact, the study by Piccolo et al. (1996) suggested that (1) the most adsorbing humic substances contained the lowest aromatic content; and (2) the larger the molecular size of the humic substances, the greater is the number of hydrogen bonding that occurs between the herbicide and the humic molecule. Furthermore, a lesser degree of stereochemical rigidity, due to a lower content of aromatic rings, allows an easier penetration of the small herbicide molecule into the inner reactive sites of the humic macromolecule, thereby favoring glyphosate sorption. Piccolo et al. (1996) have

suggested that glyphosate sorption onto humic substances may be comparable, if not higher than that observed for clay minerals in soils.

Glyphosate sorption onto soil constituents is also influenced by the amount of phosphates present in the soil (Sprankle et al., 1975; de Jonge and de Jonge, 1999). Both studies have shown that as the quantity of phosphate in the soil increased, glyphosate sorption decreased. This has been attributed to the competition for sorption sites on the soil constituents and further confirms the importance of the phosphonate group in the sorption of glyphosate.

The soil pH has also been found to influence the sorption of glyphosate (McConnell and Hossner, 1985; de Jonge and de Jonge, 1999). Generally, with increasing soil pH, the sorption of glyphosate decreases. This effect is due to the decreased interaction as the soil minerals, soil organic matter and glyphosate becomes more negatively charged. However, at normal soil pH (5-7) the effect is most likely negligible.

2.3.1.2 Factors Affecting Mobility of Glyphosate

The combined data from several soil-leaching studies indicates that glyphosate is fairly immobile in soil and therefore has very little propensity for leaching in most soils (Franz et al., 1997). In a study done by Roy et al. (1989), the mobility of glyphosate under actual field conditions in boreal forest soils from Ontario was assessed after spraying glyphosate at a rate of 2 kg a.i./ha on a sandy soil. Overall their study showed that the herbicide remained in the upper organic layer with no detectable residues of glyphosate found at greater depths; therefore the herbicide could be considered as essentially nonleachable under the conditions of this experiment. Reuppel et al. (1977)

spotted soil thin-layer plates with glyphosate and due to the low R_f (retardation factor, a measure of movement of the different compound contained within the parent compound) values for all the soil types examined concluded that the herbicide possessed little or no propensity for leaching. However, more recent studies suggest that glyphosate bound to water-soluble humic substances may be transported through the soil profile (Piccolo and Celano, 1994). Research has also shown that negligible amounts of glyphosate have been detected in surface runoff waters as a result of the herbicide binding and subsequently transported with eroded sediment (Rueppel et al., 1977, Edwards et al., 1980; Roy et al., 1989).

2.3.1.3 Factors Affecting Degradation of Glyphosate

Glyphosate degradation in the soil occurs primarily through microbial activity (Sprankle et al., 1975; Moshier and Penner, 1978). The primary metabolite of glyphosate in soil has been identified as aminomethylphosphonic acid (AMPA) (and the concurrent production of CO_2), which was also shown to undergo degradation to CO_2 at a slightly slower rate than that of glyphosate (Franz et al., 1997).

Two main pathways for glyphosate degradation have been identified, both leading to breakage of the carbon-to-phosphorus bond (Klimek et al., 2001). In the first one, glyphosate is converted to AMPA and glyoxalate by a glyphosate oxido-reductase; AMPA is either directly metabolized to methylamine and orthophosphate, or undergoes acetylation prior to the cleavage of the C-P bond. Alternatively, the initial cleavage of the C-P bond yields sarcosine, which is further converted to glycine and a C_1-unit, which is incorporated into purines and some amino acids.

The rates of glyphosate degradation can vary considerably between different soil types and this is largely due to different textural classes and the organic matter content in the soil (Nomura and Hilton, 1977, Mossier and Penner, 1978; Smith and Aubin, 1993). Degradation rates were slower in the fine textured soils and the soils with greater organic matter content (Nomura and Hilton, 1977).

Degradation is reported to occur without any lag phase and appears to be a cometabolic process under both aerobic and anaerobic conditions (Sprankle et al., 1975; Rueppel et al., 1977; Nomura and Hilton, 1977). Degradation experiments are typically carried out utilizing soil microcosms which measure the liberated ^{14}C from ^{14}C-labelled glyphosate as $^{14}CO_2$ evolves when the herbicide is metabolized by soil microorganisms (Sprankle et al., 1975: Moshier and Penner, 1978: Nomura and Hilton, 1977; Smith and Aubin, 1993). A number of soil bacteria that degrade glyphosate, such as *Pseudomonas* (Jacob et at. 1988), *Arthrobacter* (Pipke and Amrhein, 1988), *Rhizobiaceae* (Liu et al., 1991), and *Streptomyces* (Obojska et al., 1999), have been experimentally isolated. Fungal strains capable of degrading glyphosate are *Penicillium*, *Mucor* and *Fusarium* (Krzysko and Orlik, 1997). Klimek et al. (2001) isolated a *Penicillium chrysogenum* strain that metabolizes glyphosate in media containing glyphosate as the only nitrogen source.

CHAPTER 3

Site Description and Experimental Design

3.1 Soils

The soils for this study were obtained from the Manitoba Crop Diversification Center (MCDC) which is located on the south half of Section 8-11-14 west of the Principal Meridian (W.P.M.) about 3.2 kilometers north of Carberry, Manitoba. The study site consisted of a Wellwood series (silty clay loam) which is a well to moderately drained Orthic Black Chernozemic soil developed on a mantle (25 to 120 cm) of strongly calcareous, shallow, uniform, fine loamy lacustrine sediments overlaying moderately calcareous, lacustrine sediments over moderately calcareous, deep, uniform, sandy, deltaic deposits (Mills and Haluschak, 1995). These soils occur in level to very gently sloping landscapes, or in upper positions of gentle to very gentle slopes on undulating to hummocky landscapes. The study site at the MCDC study site consists of a level topography. Wellwood soils have moderate surface permeability grading to rapid permeability with depth, moderate to moderately slow surface runoff, and a low water table during the growing season. Wellwood soils are occasionally slightly eroded and are non-stony and nonsaline. They have medium available water holding capacity, medium organic matter content, and high natural fertility. In a representative profile of Wellwood soil the solum is approximately 60 cm thick. The profile is characterized by a black to

very dark grey Ah horizon, 18 to 70 cm thick; a dark brown to a brown Bm horizon, 15 to 40 cm thick with prismatic to subangular blocky structure; a yellowish brown to pale brown BC horizon, 8 to 14 cm thick, and a Cca horizon, 7 to 10 cm thick with lime accumulation and a moderately calcareous stratified, fine sand IICk horizon. Selected soil properties are given in table 3.1.

Table 3.1 Soil properties of the top 15 cm of the soils from the MCDC study site (Moulin, 1997)

pH	Field Capacity (%)	Organic Carbon (%)	Sand (%)	Silt (%)	Clay (%)	Textural Class
7.0	38	3.32	22	57	21	SiCL

3.2 Experimental Design

In 1997, the Manitoba Crop Diversification Centre (MCDC) and the Brandon research Centre (BRC) initiated a research program with the objective to define viable potato rotations that ensure sustainable land management during the potato industry expansion. The research program is unique in that it examines six different potato crop rotation systems located at one site and covers almost every aspect of crop management. The study focuses on the impact of crop rotation on plant development and crop yield, weed populations, disease incidence and severity, and soil characteristics including soil microbial communities. As such, the study is targeted towards identifying viable potato rotations that minimize yield and quality losses due to diseases and weeds, and that maintain soil quality by controlling erosion by promoting the build-up of organic matter.

The potato crop rotation study utilized a randomized complete block design with 72 research plots in total (Figure 3.1). Each plot was 21.4 m long and 12.2 m wide with adjacent plots separated by 2.1 m pathways. Treatments consisted of six rotations ranging in duration from two to four years and containing a combination of potatoes, oilseeds, cereals and legume crops. The following rotations are included in the study (Figure 3.1):

1. Potato-canola
2. Potato-wheat
3. Potato-canola-wheat
4. Potato-oat-wheat
5. Potato-wheat-canola-wheat
6. Potato-canola (underseeded to alfalfa)-alfafa-alfafa

For the purposes of this herbicide study, all rotation experiments were analyzed as three year rotations extending from 1998 to 2000.

For each of the six potato crop rotations, each crop in the rotation was grown in each year of the study, and each rotation was replicated four times (Figure 3.1, Table 3.1). All crops were managed using best management practices with respect to tillage and seeding operations, nutrient management, and weed, insect and disease control (Table 3.2, 3.3, and 3.4). The nutrient status of the soil was determined by soil tests and fertilizers were applied to meet the required needs of the crop grown. Pesticides were applied on all plots as required to minimize crop loss due to insects, weeds and diseases. Tillage operations were performed for seedbed preparation so as to minimize soil erosion and the crops were seeded at recommended depths.

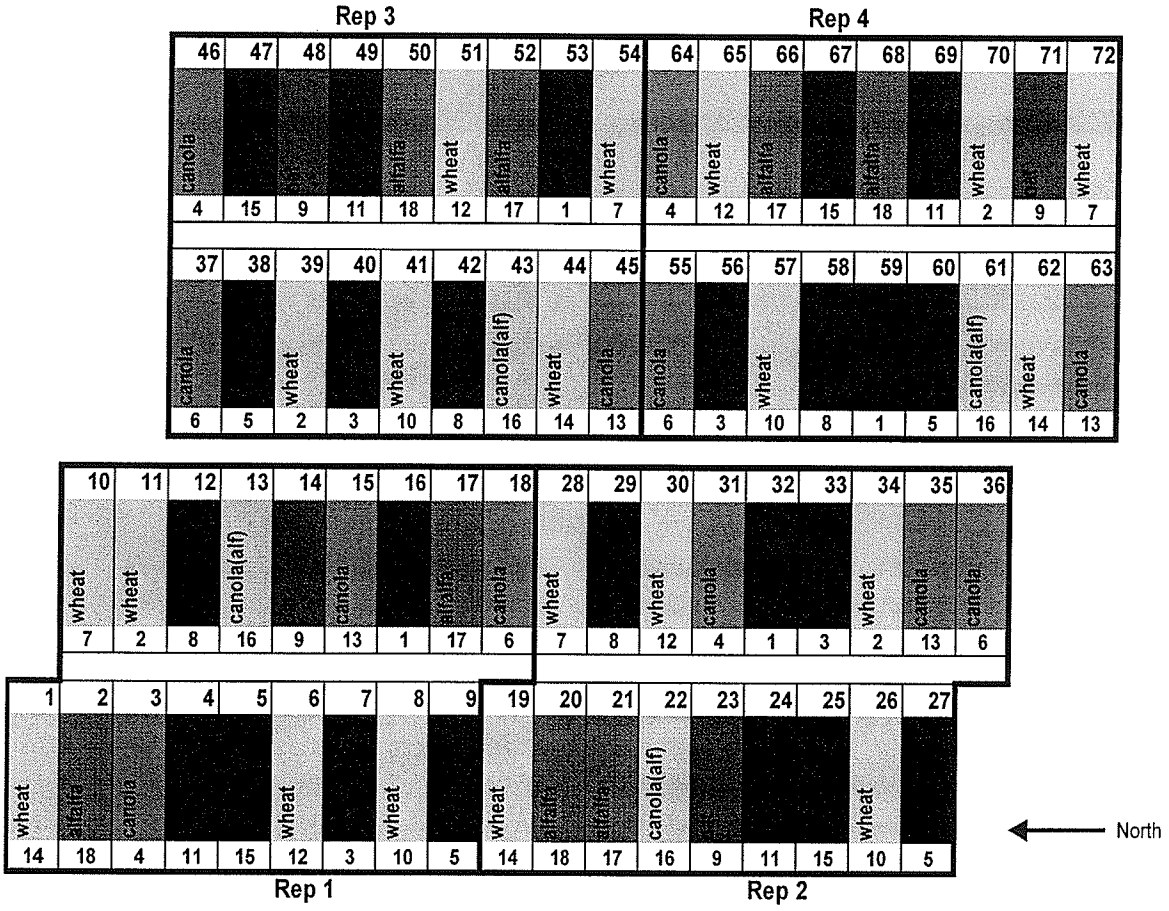

Figure 3.1 2000 field plan for the potato rotation study at MCDC, Carberry, MB. (Volkmar, et al. 2000).

Table 3.2 Overview of the potato rotation study at MCDC, Carberry, MB. (Volkmar et al., 1997).

Treatment	Phase	Plot Numbers (4 replicates/ treatment)	Crop Rotation	Crop grown in year		
				1998	1999	2000
1	1	16, 32, 53, 59	P-W*	P	W	P
2	2	11, 34, 39, 70		W	P	W
3	1	07, 33, 40, 56	P-C	P	C	P
4	2	03, 31, 46, 64		C	P	C
5	1	10, 28, 54, 72	P-C-W	P	C	W
6	2	09, 27, 38, 60		C	W	P
7	3	18, 36, 37, 55		W	P	C
8	1	08, 26, 41, 57	P-O-W	P	O	W
9	2	12, 29, 42, 58		O	W	P
10	3	14, 23, 48, 71		W	P	O
11	1	15, 35, 45, 63	P-W-C-W	P	W	C
12	2	01, 19, 44, 62		W	C	W
13	3	04, 24, 49, 69		C	W	P
14	4	06, 30, 51, 65		W	P	W
15	1	17, 21, 52, 66	P-C(A)-A-A	P	CA	A
16	2	02, 20, 50, 68		CA	A	A
17	3	05, 25, 47, 67		A	A	A
18	4	13, 22, 43, 61		A	P	CA

*Abbreviations reflect crops in crop rotations: P = potato; C = canola; W = wheat; O = oats; and CA = canola underseeded to alfalfa.

Table 3.3 Agronomic management of crops in rotation treatments at MCDC (Volkmar et al., 2000).

	Potato	Wheat	Oats	Canola	Canola/alfalfa
Tillage*	1 pass-tandem disk 1 pass-power harrow	1 pass-tandem disk	1 pass-tandem disk	1 pass-tandem disk 1 pass-harrow	1 pass-tandem disc 1 pass-harrow
Seeding Rate	15" in row and 37.5" between rows	109 kg ha^{-1}	100 kg ha^{-1}	6 kg ha^{-1}	3 kg ha^{-1}/ 7.1 kg ha^{-1}
Fertilizer**					
Nitrogen (kg ha^{-1})	140	60	60	80	80
Phosphorus (P_2O_5) (kg ha^{-1})	70	30	30	20	60
Potassium (kg ha^{-1})	30	0	0	0	0
Sulfur (kg ha^{-1})	0	0	0	15	15

*Treatments that followed potatoes in the rotation were tandem disced; all other treatments were not tilled prior to seeding. Plots were harrowed if requires for the establishment of small-seeded crops.
**Fertilizer applications are approximations as they vary from year to year according to soil tests.

Table 3.4 Typical pesticide applications (active ingredients) at MCDC (Volkmar, et al., 2000).

	Potato	Wheat	Oats	Canola	Canola/alfalfa
Herbicides	Glyphosate Paraquat/diquat Rimsulfuron	Glyphosate Clodinafop-propargyl Bromoxynil MCPA	Glyphosate Bromoxynil MCPA	Glyphosate Glufosinate-ammonium Fluazifop-p-butyl Trifluralin	Glyphosate Clethodim
Insecticides	Carbaryl Permethrin Endosulfan			Terbofos	
Fungicides	Mancozeb Chlorothalonil Metrium			Carbathin Thiram Lindane	

Table 3.5 Fertilizer inputs at MCDC (Volkmar, et al., 2000).

Treatment	Total N Input (Kg ha^{-1})			Total P Input (P_2O_5) (Kg ha^{-1})		
	1998	1999	2000	1998	1999	2000
1	150	128	146	70	70	74
2	60	42	40	25	27	30
3	150	128	146	70	70	74
4	80	40	58	20	20	20
5	150	128	146	70	70	74
6	80	40	58	20	20	20
7	60	42	40	25	27	30
8	150	128	146	70	70	74
9	60	43	50	25	27	30
10	60	42	40	25	27	30
11	150	128	146	70	70	74
12	60	42	40	25	27	30
13	80	40	58	20	20	20
14	60	42	40	25	27	30
15	150	130	130	70	70	70
16	80	6	10	60	60	100
17	none	none	none	60	none	none
18	none	none	none	20	none	none

3.2.1 Soil Sampling

Soils were sampled from the following plots for six different experiments:

a) Trifluralin extraction experiment:

Soils were sampled in September, 2000 and the plots were chosen for this study on the basis that trifluralin was applied in October, 1999 for the control of broadleaf and grassy weeds in canola crops to be grown in 2000. The plot numbers of the sampled soils were 3, 13, 15, 18, 22, 31, 35, 37, 55, 61 and 64 (Table 3.5).

b) Trifluralin sorption experiment:

Soils were sampled in September, 2000. The plots used for this study were chosen on the basis that they were potential candidates for trifluralin application. The plot numbers sampled were 3, 6, 9, 15, 18, 27, 30, 31, 33, 35, 37, 38, 40, 45, 46, 51, 55, 56, 60, 63, 64 and 65 (Table 3.5).

c) Trifluralin experiments:

The soil samples used for the trifluralin mineralization and volatilization experiments were obtained from the same bulk soil collected for the sorption experiment. In this manner, comparative analysis could be performed on the relation between soil sorption and mineralization of trifluralin. The plot numbers sampled were 3, 6, 9, 15, 18, 27, 30, 31, 33, 35, 37, 38, 40, 45, 46, 51, 55, 56, 60, 63, 64 and 65 (Table 3.5).

d) Glyphosate sorption experiment:

The soils were sampled in September, 2000. The soil samples for the sorption experiments for the rotation study were obtained from plots 1 to 36 (Table 3.2). As such, each of the six potato rotations were selected, and all crops grown in each of these six rotations. All plots were potential candidates for the application of glyphosate.

e) Glyphosate mineralization experiment:

The soil samples used for the glyphosate mineralization experiment were obtained from the composited soils representing plots 1 to 36 as collected for the glyphosate sorption experiment. Therefore, a comparative analysis could be performed on the potential relation between soil sorption of glyphosate and mineralization of the herbicide.

f) Fertilizer and humic acid experiments:

The soils used for these experiments were collected from the periphery of the plots, ensuring that fertilizers had not been applied to the soils.

3.2.2 Crop Rotation and Crop Grown

The three-year crop rotations for this study were based on the year of sampling (2000) and began with the type of crop grown in the plots from which the soil samples were obtained (Table 3.2). The trifluralin samples were obtained from four or six of the initial eighteen treatments, resulting in four and six different three-year crop rotations, while the glyphosate samples were obtained from each of the eighteen treatments and resulted

in fourteen different three-year crop rotations. For example, a two-year potato-wheat rotation that began with potato in 2000 would result in a potato-potato-wheat rotation (Table 3.2). As such, for the glyphosate studies, this method produced fourteen different crop rotations.

The soil samples for type of crop grown were categorized independently from the type of crop rotation. For example, the potato crop was part of each of the six rotations at the MCDC site, but was analyzed separately.

CHAPTER 4

Extraction of Trifluralin from a Field Soil

4.1 Abstract

Injury to sensitive crops such as cereals may occur from trifluralin residues applied for weed control in oilseed crops the previous cropping season. The objective of this study was to determine whether trifluralin residues had persisted in the soil one year after application and whether the crop rotation had an effect on the persistence of the herbicide. The herbicide was applied at a rate of 1.0 kg/ha of active ingredient in the fall of 1999 on plots that were to be seeded to canola the following year. The extractable amounts were the lowest in the potato-wheat-canola rotation (0.20 kg/ha) and highest in the alfalfa-potato canola-(underseeded to alfalfa) rotation (0.39 kg/ha). The differences in the mean values among the treatment groups were not statistically significant ($P = 0.263$). The amounts of trifluralin residues detected in the soil should not pose a threat to sensitive crops grown the year after canola.

4.2 Introduction

Trifluralin is a soil applied, preplant-incorporated herbicide commonly used in oilseed and legume crops for the control of annual grasses and broadleaf weeds (Anonymous, 1999). The herbicide residues often persist past the growing season and capable of

injuring sensitive crops such as wheat grown the following year (Gerwing and McKercher, 1992; Morrison et al., 1991). Grover et al., (1988) reported that trifluralin carryover in Western Canada typically ranges from 0.21 and 0.42 kg/ha, largely dependent on soil moisture conditions during the year of application. Field persistence of trifluralin is dependent upon many other factors such as edaphic considerations, temperature, herbicide application rates and depth of soil incorporation (Smith and Aubin, 1994). Tillage practices that thoroughly mix the soil deeper than the depth of herbicide incorporation may dilute trifluralin residues and reduce the risk of trifluralin carryover injury to rotational crops (Hartzler et al., 1989). The risk of crop injury increases with conditions that reduce crop vigour, such as seedling disease, cold soil, deep seeding, high salt concentration, soil compaction, water logged soil, drought, or low quality seed, and carryover can be higher for granular formulations than for flowable or emulsifiable concentrate formulations (Anonymous, 1999).

Morrison et al., (1989) have shown that as the amount of trifluralin residues in the soil increased, wheat emergence decreased and at the highest herbicide dosage detected (1 kg ha^{-1}) the wheat stand was reduced by 35 to 45%. They found the roots of injured plants were thicker and shorter than those of plants in untreated plots and the coleoptiles of affected plants were shorter and less translucent than those of untreated plants. However, research has shown that lower amounts of trifluralin residues in the soil environment does not subject sensitive crops to injury and therefore will not reduce yield loss (Mill et al., 1985; Hartzler et al., 1989).

4.3 Objectives of the Study

The objective of this study was to determine whether trifluralin residues could be detected in the soil one year after application and whether a three year crop rotation had an impact on the amount detected in the soil samples.

4.4 Materials and Methods

4.4.1 Site Description

The plots that were sampled for the extraction study are specified in chapter 3.

4.4.2 Trifluralin Application

Trifluralin was applied at a rate of 10.1 kg/ha as Rival 10G formulated as 10% granular trifluralin and was broadcast onto the plots with a Valmar applicator and incorporated with a 6' tandem disk on October 28, 1999. This rate corresponds to 0.846 ug of active ingredient per gram of soil (0.846 ppmw). The specific amounts applied to each plot were calculated and applied by the technical staff at the MCDC site.

4.4.3 Soil Sampling and Preparation

The soil samples were collected in September 2000 from the 12 plots that had soil-incorporated trifluralin applied on October 28, 1999. A Dutch auger was used to obtain four random samples (0-10 cm) from each plot. The depth was chosen on the basis that trifluralin be soil-incorporated to a depth of 5-8 cm (Anonymous, 1999). The samples from each plot were composited, placed in plastic bags and stored in a freezer to prevent

microbial degradation until utilized for the extraction experiment. For the extraction analysis, the samples were air-dried and homogenized using a mortar and pestle.

4.4.4 Extraction Procedures for Trifluralin

The extraction procedures were derived from methods used by Gerwing and McKercher (1992) and Corbin et al. (1994) with certain modifications applied.

A 50 g sample of air-dried soil was placed in a 250 Erlenmeyer flask and 100 ml of extractant solution was added which consisted of methanol and 2.5% glacial acetic acid, by volume. The analyses were conducted in triplicate for each experimental plot. The flasks were then placed in a platform shaker for 2 hours. The mixture was then suction filtered through a #2 filter paper into a 500 ml vacuum flask that contained 5 g of anhydrous sodium sulphate (99.0% purity, Anachemia Canada Inc. Montreal, QC. Rouses Point, NY 12979) which acts as a drying, agent using a Buchner funnel. The flask containing the soil was then rinsed with 20 ml of extractant solution and then the mixture was filtered. The extraction solution was placed in a 250 ml round-bottomed flask and evaporated to 5 ml using a rotary evaporator. An aliquot of the solution was then analyzed by gas chromatography.

4.4.5 Determining Extraction Efficiency of Trifluralin

In order to determine the extraction efficiency of the method developed for trifluralin three 50 g soil samples in 250 ml Erlenmeyer flasks were spiked with 20 ppm of analytical grade trifluralin dissolved in methanol. The soil samples were thoroughly

mixed and allowed to sit in the fume hood for 1 hr at which time they were extracted by the method outlined in 4.4.4.

4.4.6 Gas Chromatography

Trifluralin residues in soil were quantified using a Hewlett-Packard Hp 6980 gas chromatograph. The final results were quantified by HP ChemStation software. The initial oven temperature was at 70 °C and increased at a rate of 20^0 C min^{-1} reaching a final temperature of 300 °C. The capillary column was a J&W DB-5MS with a length of 30 m, a nominal diameter of 250 um and a nominal film thickness of 0.25 um operating at a maximum temperature of 325°C. The front inlet was splitless with the initial temperature of 300 °C. The carrier gas was helium at a flow rate of 70.1 ml min^{-1}. The front detector was equipped with a nitrogen-phosphorus detector (NPD) operating at a temperature of 325 °C. The detector gases were hydrogen and air with flows of 3.0 ml min^{-1} and 60.0 ml min^{-1} respectively. The retention time of trifluralin was 6 min and 51 seconds. One-microliter sample volumes were injected directly into the column through an injection port heated at 220 °C. The standard curve for analytical purposes consisted of 2, 4, 6, 8 and 10 ppm analytical grade trifluralin in methanol.

4.4.7 Statistical Analysis

The statistical analysis was accomplished by a one-way analysis of variance (ANOVA) using JMP IN software (SAS Institute, 1996). The mean comparisons analyzed were designed to account for the unequal replicates in the study and were separated according to the Fisher LSD method at alpha = 0.05.

4.5 Results

The amounts of trifluralin extracted were lowest in the potato-wheat-canola-rotation (0.20 kg/ha) and highest in the alfalfa-potato-canola (underseeded to alfalfa) rotation (0.39 kg/ha) (Table 4.1). The differences in the median values among the treatment groups were not statistically significant (P = 0.263). These amounts represent approximately 24 – 46 % of the total amounts of active ingredient of the herbicide applied the previous fall. The extraction efficiency of the method developed for trifluralin was 90 %. Therefore, all values quantified by GC can be increased by approximately 10 %.

Table 4.1 Extraction of trifluralin from soils from four crop rotations.

Crop Rotation	Trifluralin (kg/ha)
Canola-Potato-Canola	**0.23a*# (+/- 0.05)**
Wheat-Potato-Canola	**0.23a* (+/- 0.03)**
Potato-Wheat-Canola	**0.20a** (+/- 0.03)**
Alfalfa-Potato-Canola (underseeded to Alfalfa)	**0.39a*** (+/- 0.20)**

*Means of three plots followed by standard deviation.
**Means of two plots followed by standard deviation.
***Means of four plots followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way ANOVA).

4.6 Discussion

The results of this experiment have shown that trifluralin residues were detected in the soils from the experimental plots one year after application. Crop rotation did not significantly affect the amount of residue remaining in the soil environment. The rotation study was in the third year and may not have had time to modify soil factors such as organic matter input, which in turn, may have affected the persistence of the herbicide. The results may be significantly different in long-term crop rotations as the organic matter of the soil is profoundly influenced by the cropping system imposed on it (Janzen et al., 1992). In this study, the two year potato-canola rotation would have a lower contribution of organic matter input than a four year potato-wheat-canola-wheat rotation. Organic matter is the most important soil factor influencing trifluralin sorption in the soil and the stronger sorption of the herbicide may therefore lengthen the persistence in the soil environment (Peter and Weber, 1985). However, high levels of organic matter may also inactivate the herbicide (Solbakken et al., 1982; Peter and Weber, 1985). As a consequence, soil persistence does not necessarily confer toxicity on the herbicide and therefore may actually mitigate crop injury.

The similar amounts of trifluralin detected from the soil samples is a reflection that the study site was subjected to a uniform environmental conditions as well as similar timing and method of application.

The amount of trifluralin residues detected in the soil samples would not be expected to cause injury to sensitive crops grown the following year. Miller et al. (1985) have shown that trifluralin residues of 0.39 kg/ha did not confer crop injury to cotton crops. Hartzler et al. (1989) concluded that trifluralin applied at labeled rates did not pose a

serious threat to corn planted the following year. A growth chamber study by Olson and McKercher (1985) showed that trifluralin detected at 0.52 kg/ha showed reduced root dry weight of different wheat cultivars. The amounts detected at the MCDC soil samples were much less than 0.52 kg/ha and hence the plots should be suitable to prevent injury in cereal crops planted the year after the canola crops.

4.7 Summary and Conclusions

The results of this experiment have confirmed published data that trifluralin has the potential to persist in the soil environment for one year following application. There were no significant differences between the crop rotations, which may be a reflection of the short duration of the rotations, the uniform soil and environmental conditions, and uniform application timing and methods. Crop injury would not likely occur from the amounts of trifluralin residues detected in the MCDC experimental plots

CHAPTER 5

The Sorption and Desorption of Trifluralin in Soil

5.1 Abstract

Trifluralin is a soil-incorporated herbicide commonly applied for the control of broadleaf and grassy weeds in canola crops. Understanding the sorption and desorption behavior of this herbicide is important as trifluralin may persist in the soil environment past the season of application and therefore could have negative agronomic and environmental implications. The purpose of this study was to determine whether crops grown in a potato rotation and the type of rotation had an impact on the extent of trifluralin sorption and desorption in soil. Herbicide sorption and desorption was determined using standard batch equilibrium procedures.

There were no significant differences between soil organic carbon content and type of crop rotation. And the type of crop rotation did not significantly impact trifluralin sorption by soil. However, there was a statistically significant relationship between soil organic carbon content and type of crop grown in the rotation. This was also a significant impact of the type of crop grown and sorption of trifluralin in soil. Linear regression analysis suggested there was a moderate relation between soil organic carbon and trifluralin sorption by soil ($R^2 = 0.502$). The implications of these results suggests that changes in soil organic matter content input due to crops grown in a rotation potentially will influence the sorption of trifluralin in the soil.

5.2 Introduction

Research has shown that trifluralin is strongly adsorbed onto soil particles and the primary factor is the soil organic matter content (Bardsly et al., 1967; Peters and Weber, 1985; Pederson et al., 1995; Solbakken et al., 1982). Different crops are expected to contribute varying amounts of soil organic matter (SOM). For example, continuous cropping to potatoes will result in less organic matter additions to the soil than amounts added by cereals or forage legumes (Angers et al., 1999).

Trifluralin's shape, molecular size and its behavior as a nonionic compound facilitate it's binding to organic matter by van der Waals forces (Solbakken et al., 1982). The strong binding to soil particles has environmental as well as agronomic ramifications. Studies have shown that as the organic matter in soil increases, the phytotoxicity of trifluralin decreases due to the greater herbicide sorption (Helling et al., 1976; Solbakken et al., 1982). The persistence of the herbicide may also increase because of a reduced rate of degradation (Miller et al., 1975).

The strong sorption of the herbicide to the soil constituents may mitigate leaching of the herbicide through the soil profile and restrict the amount available for groundwater contamination (Miller et al., 1975; Kim and Feagle, 1998; Malterre et el., 1998; Malterre et al., 1997). Trifluralin strongly sorbed to soil particles may move off-site via water erosion (Brown et al., 1995; and Gouy et al., 1999). A study by Gouy et al. (1999) utilized simulated rainfall on experimental plots to generate a large load of eroded sediment particles. They found that very low concentrations of trifluralin were detected in runoff waters, but 90 % of the herbicide applied was adsorbed onto eroded and transported soil particles. The maximum amounts of trifluralin concentration detected by

Brown et al. (1995) in surface drainage water from an experimental field which received 275 and 456 mm of rainfall during the two growing seasons was 0.8 ug per litre suggesting the herbicide is relatively immobile in the soil environment.

Trifluralin has been detected in wind-eroded sediment, which represents one mechanism for off-site movement of the herbicide. Larney et al. (1999), found trifluralin at concentrations ranging from 312-to 353-ug kg^{-1} at heights of 10-100 cm in wind erosion samplers, which accounted for 1.4% of the herbicide applied. The soil-incorporation of the herbicide was deemed responsible for the low amounts detected.

5.3 Objectives of the Study

The objective of this study was to determine whether soil organic matter content, the type of crop rotation and the crop grown in the rotation had an impact on the sorption and desorption behavior of trifluralin in the soil environment.

5.4 Materials and Methods

5.4.1 Site Description

The plots from which the soil samples for the experiment were collected are outlined in Chapter 3.

5.4.2 Soil Sampling and Preparation

Soil samples were collected in September 2000 from plots that contained a canola crop in the rotation with the exception of the canola-underseeded to alfalfa rotations as trifluralin would not be applied to these plots. Four random samples (0-10 cm) per plot

were taken using a Dutch auger. These samples were composited and placed in plastic bags and stored at 4^0C until utilized for the experiment at which time they were air-dried for 24 hours and sieved through a 2mm mesh.

5.4.3 Soil Organic Carbon Analysis

Soil samples (5 g) from each plot were analyzed for organic-carbon. Inorganic carbon was removed prior to organic carbon measurement by adding 10 ml of 6M HCl in distilled water to the soil and heating the soil slurry on a hot plate for 10 minutes (Tiessen et al., 1983). The samples were rinsed with 240 ml of distilled water to remove the inorganic ions present. Soil organic carbon was determined by dry combustion of 0.12 g of oven-dried soil with a Leco model CHN 600 C and N determinator (Nelson and Sommers, 1982).

5.4.4 Sorption and Desorption Procedures

Batch-equilibrium experiments were conducted in duplicate to quantify the sorption/desorption of trifluralin in the soil. The herbicide stock solution was prepared by adding ring-UL-^{14}C-trifluralin (sp. act. 16.8 mCi/mmol-1; Sigma-Aldrich Canada Ltd. Oakville, On.) and commercial grade Treflan (Dow AgroSciences) to a 0.01 M $CaCl_2$ (anti-dispersing agent) solution. Each ml of the herbicide solution contained 35 Bq of ^{14}C-trifluralin and 1 ug of Treflan. The herbicide solution (15 ml) was added to 5 g of soil in 50 ml Teflon centrifuge tubes to simulate field application rates of 1.1 kg/ha of active ingredient per hectare. The field application rate added to the soil samples was calculated by determining the amount of the label rate of the herbicide that would be in the top 10

cm of the field soil after application. The tubes were then placed in a rotary shaker for 24 hours in the dark and at room temperature to reach equilibrium. The soil slurries were centrifuged at 10,000 RPM for 10 min and 1 ml of the supernatant was sub sampled in duplicate to quantify the concentration of the herbicide remaining in solution. The supernatant was placed in a 15 ml scintillation vial and 10 ml of Scintisafe, 30% (Fisher Scientific, Fairlawn, New jersey). The vials were allowed to sit in a darkened room for 24 hours, after which the radioactivity was quantified by using a Beckman LS 7500 liquid scintillation counter (LSC). The amount of radioactivity detected was subtracted from the amount initially applied to give the amount sorbed by the soil constituents and was given as a percentage of the original amount applied. The sorption distribution coefficient, Kd [ml g^{-1}] was calculated assuming linear partitioning (1/n=1) according to the following equation:

$$Kd = Cs/Ce \qquad \textbf{Equation 5.1}$$

where Cs = the concentration of the herbicide sorbed by the soil at equilibrium [mg g^{-1}] and Ce = the concentration of the herbicide in solution at equilibrium [mg ml^{-1}]. Greater Kd values indicate greater herbicide sorption relative to smaller Kd values.

For the desorption procedures, an additional 5 ml of the supernatant was removed and replaced by adding 7 ml of 0.01 M of $CaCl_2$ solution to the centrifuge tubes. The batch-equilibrium procedures for the sorption experiment were repeated and the amount of ^{14}C-trifluralin desorbed was quantified by LSC. The amount of the herbicide desorbed from the soil was calculated by the following method. The herbicide remaining in 8 ml of solution after the sorption experiment was recorded (ug/ml). The amount remaining in the solution after the desorption experiment was recorded (ug/ml) and the amount of

herbicide in solution after the sorption experiment was subtracted from this amount. All desorption values were given as a percentage of the original herbicide in solution. The remaining portion of trifluralin was assumed to be sorbed onto the soil constituents. The LSC results for both the sorption and desorption measurements for each sample were corrected by subtracting the background radiation.

5.4.5 Statistical Analysis

The data generated in the sorption and desorption experiments were subjected to a one-way analysis of variance (ANOVA) using JMP IN software (SAS Institute, 1996) and linear regression analysis using the SigmaPlot 2000 software (1986-2000 SPSS Inc.). The mean comparisons analyzed were designed to account for the unequal replicates in the study and were separated according to the Fisher LSD method at alpha = 0.05.

5.5 Results

The results of the sorption experiment demonstrated that the herbicide was strongly sorbed onto the soil constituents regardless of the crop rotation or crop grown (Table 5.1 and 5.2). All soil samples exhibited trifluralin sorption greater than 98% while less than 1% of the herbicide was desorbed from the soil.

The amount of soil organic carbon (SOC) varied slightly between the different crop rotations with wheat-potato-wheat rotation containing the lowest amounts (2.88%) and the wheat-potato-canola rotation containing the highest amounts (3.66%) (Table 5.1). There was a statistically significant difference among the treatment groups ($P = 0.044$). The SOC amounts values were significantly higher in the potato-wheat-canola rotation

with respect to the other crop rotations in the study. The lowest sorption value was in the potato-canola-potato rotation (98.85%) and the highest in the potato-wheat-canola rotation (99.19%). The differences in the mean values of the rotational groups were not statistically significant (P = 0.179). The Kd values were the lowest in the canola-wheat-potato rotation (284) and highest in the potato-wheat-canola rotation (375) (Table 5.1). The differences in the mean Kd values for the rotational groups were not statistically significant (P = 0.207).

The desorption of trifluralin was the lowest in the potato-canola-potato rotation (0.11%) and the highest in the canola-wheat-potato rotation (0.44%) (Table 5.1). The differences in the mean values among the rotational groups were not statistically significant (P = 0.119).

Table 5.1 Sorption and desorption of trifluralin in relation to crop rotation. Kd [ml g^{-1}] was determined by batch equilibrium experiments.

Rotation	%Organic-C	% Sorption	Kd	% Desorption
Canola-Potato-canola	3.43b*# (+/ 0.132)	99.05a (+/- 0.108)	318a (+/- 38)	0.29a (+/- 0.213)
Potato-canola-potato	3.38b** (+/- 0.267)	98.85a (+/- 0.385)	278a (+/- 61)	0.11a (+/- 0.080)
Wheat-potato-wheat	2.88b* (+/- 0.561)	99.16a (+/- 0.130)	366a (+/- 55)	0.27a (+/- 0.106)
Potato-wheat-canola	3.60b* (+/- 0.294)	99.19a (+/- 0.162)	375a (+/- 68)	0.23a (+/- 0.108)
Canola-wheat-potato	3.51b* (+/- 0.189)	98.92a (+/- 0.217)	284a (+/- 68)	0.44a (+/- 0.139)
Wheat-potato-canola	3.66a** (+/- 0.240)	99.14a (+/- 0.064)	348a (+/- 25)	0.29a (+/- 0.11)

*Means of four replicates followed by standard deviation.
**Means of three replicates followed by standard deviation.
#Means in the same column followed by the same letter are not statistically significant at $P < 0.05$ (One-way ANOVA).

The amount of soil organic carbon was the lowest in the potato plots (3.09%) and highest in the plots containing the wheat crops (3.60%) (Table 5.2). There was a statistically significant difference among the treatment groups (P = 0.029). The SOC was significantly higher in the soil cropped to wheat than in the soil cropped to canola and potato. The soil sorption of trifluralin was lowest in the potato crops (98.98%) and highest in the wheat crops (99.16%). There was a statistically significant difference among the different crops (P = 0.032). The sorption of the herbicide was significantly higher in the soil cropped to wheat than the soils cropped to canola and potato. The Kd values were lowest in the potato crop (281) and highest in the wheat crop (367) (Table 5.2). The mean values of the different crops were not statistically significant (P = 0.052). The Kd values for the sorption values changed a great deal even though the sorption values did not which may account for the fact that sorption was statistically significant and the Kd values were not and may be the result of the different calculations used for the % sorbed.

The desorption values were lowest in the wheat crop (0.27%) and highest in the canola crop (0.34%) (Table 5.2). The mean values of the different crops were not statistically significant (P = 0.992).

Table 5.2 Sorption and desorption of trifluralin in relation to crop grown. Kd [ml g^{-1}] was determined by batch equilibrium experiments.

Crop	% Organic-C	% Sorption	Kd	% Desorption
Potato	**3.09 a*#**	**98.89 a*#**	**281a**	**0.30a**
	(+/- 0.50)	**(+/- 0.27)**	**(+/- 75)**	**(+/- 0.22)**
Canola	**3.52 a****	**99.13 a****	**347a**	**0.34a**
	(+/- 0.22)	**(+/- 0.13)**	**(+/- 51)**	**(+/- 0.15)**
Wheat	**3.60 b*****	**99.16b*****	**367a**	**0.27a**
	(+/- 0.23)	**(+/0.13)**	**(+/- 55)**	**(+/- 0.09)**

*Means of seven replicates followed by standard deviation.
**Means of eleven replicates followed by standard deviation.
***Means of four replicates followed by standard deviation.
#Means in the same column followed by the same letter are not statistically significant at $P < 0.05$ (One-way ANOVA).

The linear regression analysis of the relation between soil organic carbon and Kd values for all the sampled plots showed an R^2 of 0.502 (Figure 5.1). This suggests a moderate relationship between sorption of trifluralin and soil organic matter content.

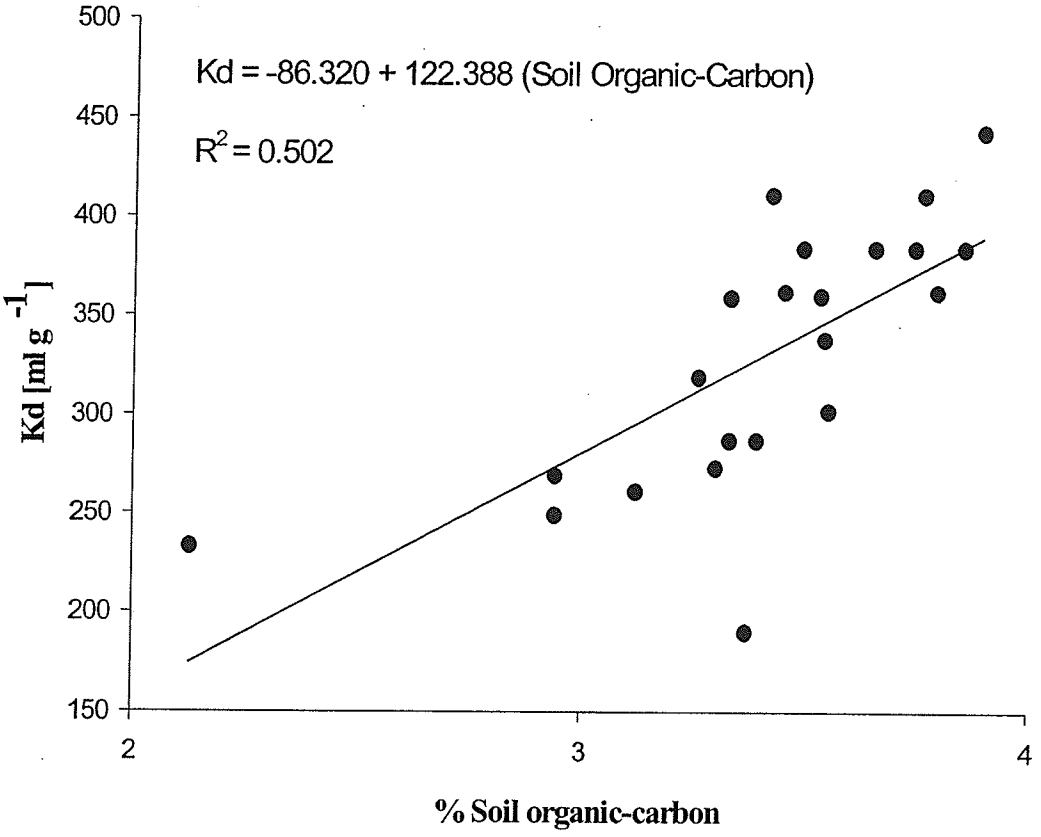

Figure 5.1 Relation between organic-carbon and trifluralin sorption by soil (Kd).

5.6 Discussion

The results of the sorption and desorption experiments showed that trifluralin is tightly and irreversibly bound to the soil constituents irrespective of the type of crop rotation or the type of crop grown in the rotation. Trifluralin is commonly applied in the fall or the spring and the strong sorption assures that the herbicide remains in the soil for the effective control of broadleaf and grassy weeds.

The crop rotation did not significantly affect trifluralin sorption. The crop rotation study was in the third year and the rotation may not have been in operation long enough to have had a noticeable impact on the soil properties, in particular the organic matter content. The two-year potato-canola rotation would normally produce less soil organic matter than a four-year potato-wheat-canola-wheat rotation. Therefore, sorption behavior of trifluralin in soil may differ after four or more years into the rotation study.

There was a significant difference between soil organic carbon content and type of crop grown. The type of crop grown significantly influenced trifluralin sorption in soil. The least amount of sorption occurred in the plots cropped to potato and this crop also produced the least amount of organic carbon in the soil. These results are in line with the fact that soil organic matter content affects the sorption of trifluralin in the soil environment. As the amounts of SOC increases, the phytotoxicity of the herbicide decreases (Solbakken et al., 1982). Consequently, weed control efficacy may be reduced as well as crop injury. The strong sorption of the herbicide may also indicate a reduced rate of degradation and therefore may increase the persistence in soil (Miller et al., 1975).

The strong sorption of trifluralin would mitigate any leaching of the herbicide through the soil profile. This has implications for the study site as the Assiniboine Aquifer, which

is a vital source of drinking water for the surrounding community, is located at a depth of 1.7 to 5.2 meters below ground level (Mills and Haluschuck, 1995). The strength of sorption of trifluralin by soil within the experimental plots suggests that there is a negligible threat of the herbicide leaching of the into the aquifer.

Trifluralin has been detected in wind-eroded sediment and therefore represents one mechanism for off-site movement of the herbicide (Larney et al., 1999). Consequently, wind-eroded particles may move off-site at the MCDC location, particularly in the potato crop plots as very little residue is left on the soil surface after harvest.

The MCDC experimental site is level and therefore it is unlikely that the herbicide would move off-site via water erosion.

5.7 Summary and Conclusions

Trifluralin became very strongly sorbed onto the soil constituents from all of the soil samples obtained from the study site. This fact has wider implications for crop injury and movement from the target site. The herbicide residues strongly sorbed onto the soil constituents may reduce the phytotoxicity of the herbicide for sensitive crops grown in the rotation the following year despite the fact that persistence may be increased. Groundwater contamination of the herbicide may be negligible, however the herbicide may move from the target site via wind erosion. The eroded soil particles may be transported to surface waters or other land surfaces and cause unwanted trifluralin residues in those environments.

CHAPTER 6

Mineralization and Volatilization of Trifluralin from a Field Soil

6.1 Abstract

The study of trifluralin persistence in soil is important because herbicide residues may cause crop injury to cereals grown the following year in rotation. The purpose of this study was to examine whether crop rotation and type of crop grown had an impact on trifluralin mineralization and volatilization rates in the soil environment. Results from soil microcosm experiments indicated that trifluralin mineralization amounts were always less than 3% of the amount of the trifluralin applied. Crop rotation did not have a significant effect on trifluralin mineralization however the type of crop grown in the rotation had a significant effect on the amounts mineralized. The half-life of the herbicide in the soil samples was the shortest in the potato-canola-potato rotation and the longest in the potato-wheat-canola- rotation and were not significantly different. The half-life of the herbicide was shortest in the potato crop and longest in the wheat crop and were not significantly different. Volatilization of trifluralin was lower than 4% in all soil samples and the differences were not significant among the different crop rotations and the type of crop grown.

6.2 Introduction

After trifluralin is applied to the soil there are three major pathways of herbicide losses from the soil environment: biodegradation (including mineralization), volatilization, and photodecomposition. Photodecomposition of trifluralin is probably a minor dissipation method under field conditions since the herbicide is incorporated into the soil soon after application (Messersmith et al., 1971). Research has shown that trifluralin has the capacity to volatilize from the soil environment after application (Bardsley et al., 1967; Savage and Barrentine, 1969; Harvey, 1974; Spence and Cliath, 1974). Volatilization of trifluralin is related to soil water content as volatilization rates were found to be much higher in a moist soil than a dry soil (Harvey, 1974; Spence and Cliath, 1974). Soil organic matter content also influences trifluralin volatilization from soil (Bardsley et al., 1967). Volatilization losses decreased as the soil organic matter increased, purportedly due to the greater soil sorption of the herbicide. Soil temperature also has an impact on trifluralin volatilization. Savage and Barrington, (1969) found that significantly more trifluralin was lost as a vapor at 40°C than at 30°C. The depth of soil incorporation also influenced the amount of trifluralin volatility from the soil (Savage and Barrentine, 1969; Spencer and Cliath, 1974). The greatest amount of volatilization occurred from surface applications of the herbicide and the lowest amount when incorporation was at the 7.5 cm depth. Higher surface soil temperatures and the higher rates of air exchange under field conditions would account for this behaviour (Helling, 1976; Spencer and Cliath, 1974).

A number of studies, under field and laboratory conditions, have reported that soil microbes degrade trifluralin (Messersmith et al., 1971; Parr and Smith, 1973; Probst et al., 1975; Carter and Camper, 1975; Wheeler et al., 1975; Golab et al., 1979). A two-stage, first-order kinetics model has been used for predicting the dissipation of the trifluralin (Lafleur et al., 1980). The initial disappearance from soil occurs relatively rapidly (first stage half-life) but several years may be required for complete disappearance of the herbicide (second stage half-life). The herbicide appears to degrade in soils via aerobic and anaerobic pathways (Helling, 1976). A variety of soil microorganisms have been shown to degrade trifluralin including the bacterial species *Pseudomonas* and the fungal species *Paecilomyces* (Carter and Camper, 1975; and Messersmith et al., 1971).

Field and laboratory studies have shown that trifluralin is persistent in the soil (Helling, 1976; Wheeler et al., 1979; Berger et al., 1996). Golab et al. (1979) isolated and identified 28 transformation products of ^{14}C-trifluralin and none exceeded 3% of the initially applied herbicide. Breakdown of ^{14}C-trifluralin to CO_2 by soil microorganisms in a laboratory incubation experiment accounted for a 3% and 5% of total loss of ^{14}C-activity in a sandy loam and a silty clay soil respectively (Messersmith et al., 1971). Wheeler et al. (1979) obtained similar results in their laboratory experiments. After 83 days, trifluralin loss through conversion to $^{14}CO_2$ ranged from 2.5 to 3.1% in a silty clay loam soil and from 1.4 to 2.0% in a sandy loam soil. A field study by Gerwing and McKercher (1992) detected trifluralin residues in the field one year after application. Corbin et al., (1994) found low levels of trifluralin present (0.06 - 0.14 kg/ha) 30 months after the last trifluralin application. These figures translate into the half-life of trifluralin

ranging from 8.7 months to 14.9 months. The field persistence of trifluralin is dependent upon many factors such as edaphatic conditions, rainfall, differences in herbicide application dates and rates, method of application, and methods and depth of incorporation in the field (Smith and Aubin, 1994).

The persistence of trifluralin residues in the soil may result in crop injury to other rotational crops during the following season such as cereals (Morrison et al., 1989; Hartzler et al., 1989; Morrison et al., 1991; Nawolsky et al., 1992; Gerwing and McKercher, 1992; Corbin et al., 1994). Corn is sensitive to higher than field application rates and damage to wheat can occur if the crop is seeded into land that has been previously treated with trifluralin products and has received abnormally low amounts of precipitation during the growing season (Anonymous, 1999).

6.3 Objective of the Study

The objectives of this study were to determine whether crop rotation and type of crop grown had an impact on trifluralin mineralization, half-life, and volatilization rates in the soil environment.

6.4 Materials and Methods

6.4.1 Site Description

The field plots sampled for this experiment are specified in Chapter 3.

6.4.2 Soil sampling and Preparation

The soil samples were collected in September, 2000. A Dutch auger was used to obtain four random samples per plot from the 0 to 10 cm depth of the soil. After each plot was sampled, the auger was rinsed in a bleach solution to prevent microbial contamination between plots. The samples from each plot were composited and placed in plastic bags. The samples were stored at 4°C until utilized for the experiment.

6.4.3 Microcosm Apparatus

The microcosm apparatus for the rotation study consisted of 2-liter Mason jars with screw top lids. A 100 ml beaker containing 50 g of soil was placed in the jar. To the jars were added a 50 ml beaker containing 0.5 g of MTO-Ambersorb 563 (Supelco, Bellefonte, PA.) to trap any volatile ^{14}C-trifluralin, and a 15 ml scintillation vial containing 5 ml of 1 M NaOH solution to trap the $^{14}CO_2$ evolved by microbial respiration. Prior to herbicide application, the jars were incubated at 20°C in the dark for two weeks to increase the metabolic activity of the soil microorganisms.

A 1 ml portion of a solution containing 2.0 uCi of ^{14}C-ring labeled trifluralin (sp.act. 16.8 mCi mmol-1; Sigma-Aldrich Canada Ltd. Oakville, On.) was added drop wise by pipette to each soil. This was the equivalent of a recommended field application rate of 1.0 kg/ha of active ingredient of granular trifluralin. This corresponded to a disintegrations per minute (DPM) count of 4.87 x 10^6 per ml of herbicide solution.

trifluralin. The jars were incubated for 168 days in environmental growth chambers at 20°C in the dark. At day 70, 2 ml of water was added to the soil samples restoring them to field capacity by adding water to obtain a predetermined weight.

6.4.5 Monitoring Trifluralin Mineralization

The 15 ml scintillation vial containing NaOH was used to trap any $^{14}CO_2$ evolved during the experiment. For each of the soil samples, the sealed jars created a closed environment which allowed for the containment and trapping of the mineralization of the radiolabeled compound. These vials were changed at regular intervals over duration of the experiment (168 days). During the first three weeks they were changed every three days as the initial microbial activity and degradation proceeded more rapidly at this time than later in the experiment. The vials were then changed every two weeks for the remaining duration of the experiment.

After the traps were removed from the microcosms, 8 ml of Scintisafe was added directly to each vial. The vials were allowed to sit in the dark at room temperature for 24 hours before the radioactivity was quantified by a Beckman LS 7500 scintillation counter. The level of radioactivity per trap was given as disintegrations per minute (DPM). These values were then used to calculate the amount of $^{14}CO_2$ that had evolved during the trap's placement in the soil microcosm. The amount of $^{14}CO_2$ evolved was calculated as a percentage of the original amount of ^{14}C-trifluralin applied.

6.4.6 Volatilization of Trifluralin

The 0.5 g of Ambersorb placed in the microcosm trapped the amount of ^{14}C-trifluralin that volatilized during the course of the experiment. The cumulative amount volatilized was quantified at the cessation of the experiment.

A sub sample of 0.1 g of Ambersorb and 0.2 g of cellulose was placed in a COMBUSTO-CONE (Packard Instrument Co. Meriden, CT) and then 0.3 ml of COMBUSTAID (Packard Instrument Co.) was added. This was then placed in a Packard Oxidizer, Model 306. The burn time was set at 1.5 minutes. CARBO-SORB (5 ml) (Packard Instrument Co.) was used to trap the volatile ^{14}C-trifluralin. The oxidizer added 15 ml of Scintisafe, 30% (Fisher Scientific, Fairlawn, New Jersey) for quantification of volatile trifluralin by LSC. The Packard Oxidizer was calibrated by quantifying a standard amount of radiolabeled compound.

6.4.7 Mathematical and Statistical Analysis

The mineralization activity of the ^{14}C-trifluralin was conducted by measuring the ^{14}C that evolved as carbon dioxide and calculating it as a percent of the radioactivity that was initially added to the soil samples. A first order curve fitting was performed by using SigmaPlot 2000 software (1986-2000 SPSS Inc.) and the following equation.

$$A_t = A_F (1-e^{-K_F t}) \qquad \textbf{Equation 6.1}$$

Where A_t = percent degradation at time t (days), A_F = percent of added ^{14}C that has evolved at time infinity for the first order curve, and K_F = the degradation rate constant (days^{-1}) for the first order curve.

The K values that were determined by curve fitting were then used to calculate the half-life for trifluralin in each of the sampled plots. The half-life is calculated based on the percent of the chemical that was mineralized, therefore the half-life presented here is an indication of the time it takes for 50% of the mineralizable fraction to be mineralized. The following equation was used.

$$t^{1/2} = \ln 2/K \qquad \textbf{Equation 6.2}$$

JMP In software (1996 SAS Institute Inc.) was used to perform a one-way analysis of variance (ANOVA) on the final cumulative degradation values of trifluralin in the soil samples. The mean comparisons analyzed were designed to account for the unequal number of replicates in the study and were separated according to the Fisher LSD method at alpha = 0.05.

6.5 Results

6.5.1 Mineralization Study

The mineralization of trifluralin proceeded without a lag phase in all of the soil samples (Figure 6.1). For all plots the average mineralization of trifluralin, as quantified by the amount of $^{14}CO_2$ evolved after 168 days, was less than 3% of the total applied. After approximately 12 weeks the rate of mineralization began to slow down noticeably in all of the soils. At this time $^{14}CO_2$ production had reached more than half of the total amount measured at 168 days. An slight increase in the mineralization rate was observed at 80 days and is attributed to the addition of 1 ml of water to all of the samples.

After 20 days, the mineralization of trifluralin was less than 0.65% of the total applied radioactivity in soils from the crop rotations (Table 6.1). The lowest amount of

mineralization in soil occurred in the wheat-potato-wheat rotation (0.52%) while the greatest amount occurred in the potato-canola-potato rotation (0.74%). There was not a significant difference in the amount mineralized among soils from the different rotations (P = 0.156).

Table 6.1 Mineralization of trifluralin from soil from six crop rotations after 20 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop rotation	$^{14}CO_2$ Evolved (%)
Canola-potato-canola	**0.54a*# (+/- 0.12)**
Potato-canola-potato	**0.74a** (+/- 0.21)**
Wheat-potato-wheat	**0.52a* (+/- 0.05)**
Potato-wheat-canola	**0.50a* (+/- 0.02)**
Canola-wheat-potato	**0.61a* (+/- 0.11)**
Wheat-potato-canola	**0.62a** (+/- 0.15)**

*Means of four replicates followed by standard deviation.
**Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at P < 0.05 (One-way AVOVA).

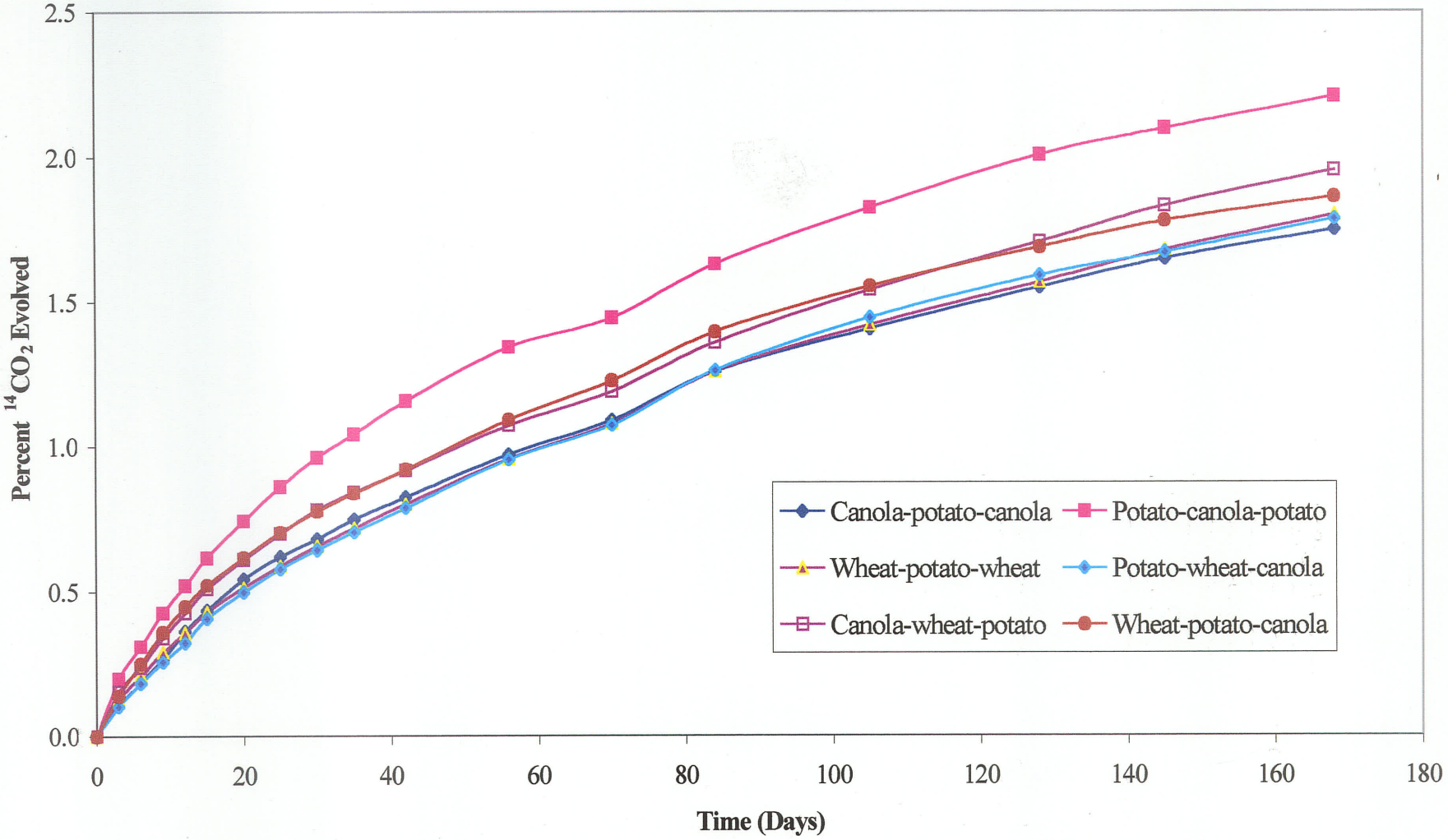

Figure 6.1 Trifluralin mineralization in soils from six crop rotations. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

After 105 days, which is the approximate length of the Manitoba growing season, the mineralization of trifluralin ranged from 1.43% to 1.61% of the applied radioactivity in the crop rotations (Table 6.2). The lowest amounts occurred in soils from the wheat-potato-wheat rotation while the largest amount occurred in soils from the potato-canola-potato rotation (1.82%). The trend is consistent with the rates occurring after 20 days of incubation. The differences in mineralization amounts among the treatment groups were not statistically significant (P = 0.100).

Figure 6.2 Mineralization of trifluralin from soil from six crop rotations after 105 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop rotation	$^{14}CO_2$ Evolved (%)
Canola-potato-canola	**1.41a*# (+/- 0.11)**
Potato-canola-potato	**1.82a** (+/- 0.37)**
Wheat-potato-wheat	**1.42a* (+/- 0.06)**
Potato-wheat-canola	**1.44a* (+/- 0.06)**
Canola-wheat-potato	**1.54a* (+/- 0.15)**
Wheat-potato-canola	**1.55a** (+/- 0.28)**

*Means of four replicates followed by standard deviation.
**Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way AVOVA).

After 168 days the lowest mineralization of trifluralin occurred in the canola-potato-canola rotation (1.75%) and the highest amount occurred in soils from the potato-canola-

potato rotation (2.24%) (Table 6.3). Crop rotation did have a significant effect on the mineralization of trifluralin in each of the field soils samples at the cessation of the experiment (P = 0.037). Trifluralin mineralization was significantly lower in the canola-potato-canola, wheat-potato-wheat, potato-wheat-canola and wheat-potato-canola rotations and each rotation was significantly different from each other.

Table 6.3 Mineralization of trifluralin in soil from six crop rotations after 168 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop rotation	$^{14}CO_2$ Evolved (%)
Canola-potato-canola	**1.75b*# (+/- 0.10)**
Potato-canola-potato	**2.24a** (+/- 0.36)**
Wheat-potato-wheat	**1.80c* (+/- 0.06)**
Potato-wheat-canola	**1.78d* (+/- 0.02)**
Canola-wheat-potato	**1.94a* (+/- 0.13)**
Wheat-potato-canola	**1.89e** (+/- 0.06)**

*Means of four replicates followed by standard deviation.
**Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at P < 0.05 (One-way AVOVA).

The mineralization of trifluralin in soil from three crops after 20 days ranged from 0.086% to 0.113% of the total applied radioactivity (Table 6.4). There was no significant difference in the mineralization amount in the soil within the different crops grown (P = 0.245).

Table 6.4 Mineralization of trifluralin in soil from three crops after 20 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop	$^{14}CO_2$ Evolved (%)
Potato	**0.113a*# (+/- 0.03)**
Canola	**0.099a** (+/- 0.02)**
Wheat	**0.086a*** (+/- 0.03)**

*Means of seven replicates followed by standard deviation.
**Means of eleven replicates followed by standard deviation.
***Means of four replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way ANOVA).

After 105 days, which is the approximate length of a Manitoba growing season, the mineralization of trifluralin ranged from 1.42% in the wheat crop to 1.78% in the potato crop (Table 6.5). The differences in the mineralization amounts of trifluralin in soil were not significant ($P = 0.093$).

Table 6.5 Mineralization of trifluralin in soil from three crops after 105 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop	$^{14}CO_2$ Evolved (%)
Potato	**1.78a* (+/- 0.28)**
Canola	**1.52a** (+/- 0.16)**
Wheat	**1.42a*** (+/- 0.06)**

*Means of seven replicates followed by standard deviation.
**Means of eleven replicates followed by standard deviation.
***Means of four replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way ANOVA).

After 168 days, mineralization of trifluralin was the highest in the potato crop (2.07%) and lowest in the canola and wheat crops (1.80%) (Table 6.6). The type of crop grown produced a significant difference in the mineralization of trifluralin (P = 0.026). Soils cropped to potato were significantly higher, relative to soils cropped to canola and wheat.

Table 6.6 Mineralization of trifluralin in soil from three crops after 168 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop	$^{14}CO_2$ Evolved (%)
Potato	**2.07a#* (+/- 0.28)**
Canola	**1.80b** (+/- 0.16)**
wheat	**1.80b*** (+/- 0.06)**

*Means of seven replicates followed by standard deviation.
**Means of eleven replicates followed by standard deviation.
***Means of four replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way ANOVA).

The half-life of readily mineralizable trifluralin from the field soils was the shortest in the potato-canola-potato rotation (42 days) and the longest in the potato-wheat-canola rotation (56 days) (Table 6.7). There was not a significant difference in the half-life of trifluralin in the soil within the different crop rotations (P = 0.568).

Table 6.7 Trifluralin half-life in soil from six crop rotations (Days).

Crop rotation	Half-life (Days)
Canola-potato-canola	**50a*# (+/- 17)**
Potato-canola-potato	**42a** (+/- 12)**
Wheat-potato-wheat	**54a* (+/- 11)**
Potato-wheat-canola	**56a* (+/- 11)**
Canola-wheat-potato	**47a* (+/- 11)**
Wheat-potato-canola	**43a** (+/- 6)**

*Means of four replicates followed by standard deviation.
**Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way AVOVA).

The half-life of readily mineralizable trifluralin from the field soils was the shortest where the potato crop was grown (45 days) and the longest in the wheat crop (54 days) (Table 6.8). There was not a significant difference in the half-life of trifluralin in the soil within the different crops grown ($P = 0.420$).

Table 6.8 Trifluralin half-life in soil from three crops (Days).

Crop	Half-life of Trifluralin (Days)
Potato	**45a*# (+/- 10.7)**
Canola	**48a** (+/- 12.5)**
Wheat	**54a*** (+/- 10.9)**

*Means of seven replicates followed by standard deviation.
**Means of eleven replicates followed by standard deviation.
***Means of four replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way ANOVA).

6.5.2 Volatilization Study

Combustion of the Ambersorb compound revealed that ^{14}C-trifluralin had volatilized from the soil and into the confined atmosphere of the microcosm jars. The amounts of the herbicide volatilized ranged from 2.29% of the initially applied radioactivity in the wheat-potato-canola rotation to 4.00 % in the wheat-potato-canola rotation (Table 6.9). There was no significant difference in the volatilization rates among the treatment groups ($P = 0.335$).

Table 6.9 Volatile ^{14}C-trifluralin recovered from the Ambersorb traps for the soil samples from all of the crop rotations. Calculated as the cumulative percent of added radioactivity recovered in the Ambersorb.

Crop rotation	^{14}C-Trifluralin Recovered (%)
Canola-potato-canola	**3.05a*# (+/- 0.89)**
Potato-canola-potato	**2.90a** (+/- 0.68)**
Wheat-potato-wheat	**2.29a* (+/- 0.60)**
Potato-wheat-canola	**2.81a* (+/- 1.20)**
Canola-wheat-potato	**3.48a* (+/- 0.70)**
Wheat-potato-canola	**4.00a** (+/- 0.60)**

*Means of four replicates followed by standard deviation.
**Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way AVOVA).

The amounts of volatile trifluralin detected from the Ambersorb traps for the soil samples from the different crops ranged from 2.29% in the wheat crop to 3.29% in the

canola crop (Table 6.10). There was not a significant difference in the volatilization rates among the different crops grown (P = 0.255).

Table 6.10 Volatile ^{14}C-trifluralin recovered from the Ambersorb traps for the soil samples from each of the crops in rotation. Calculated as the cumulative percent of added radioactivity recovered in the Ambersorb.

Crop	^{14}C-Trifluralin Recovered (%)
Potato	**3.19a*# (+/- 0.71)**
Canola	**3.29a* (+/- 1.21)**
Wheat	**2.29a** (+/- 0.60)**

*Means of seven replicates followed by standard deviation.
**Means of eight replicates followed by standard deviation.
#Means followed by the same letters are not significantly different at $P < 0.05$ (One-way ANOVA).

6.6 Discussion

The results of this study show that trifluralin undergoes microbial degradation in the soil environment. Mineralization of the herbicide occurred without a lag phase indicating that a microbial population capable of metabolizing the herbicide resided in the soil. A noticeable increase in the mineralization rate occurred after day 70 and can be attributed to the addition of 2 ml of water to the soil samples. The amount of mineralization that occurred after 168 days accounted for less than 3% of the applied radioactivity. A number of factors could account for the low microbial metabolism of the herbicide (Rueppel et al., 1977). Sorption of trifluralin by the soil constituents is very strong and therefore the compound may not have been very accessible to the soil microbes (Helling, 1976). The microbial population that was currently residing in the soil may not have been composed of species, such as *Pseudomonas*, which are largely responsible for trifluralin

degradation. Concomitantly, *Psuedomonas* are aerobic bacteria, which oxidize substrates such as pesticides to CO_2 (Prescott et al., 1993). *Pseudomonades* are classified as rhizobacteria, which form close associations with the rhizosphere, or the area immediately surrounding the plant roots (Paul and Clarke, 1996). Microbial counts increase significantly in the rhizosphere because of the wide range of organic materials provided by the roots that act as substrates for the microorganisms. The soil microcosms in this study do not contain growing plants and therefore are devoid of roots. As a result, the number of *Pseudomonas* colonizing the soil environment may be much less than if the soil were in a natural cropping environment. Although the microcosm environment may have contained optimal moisture and temperature conditions, it did not resemble an actual field environment. As such, the results in this study may not be directly indicative of what may occur in a field environment after applications of trifluralin.

There was a significant difference in mineralization among the three types of crop grown in the field plots. However, since less than 3% was mineralized after 168 days the statistical significance may be questionable.

The Ambersorb traps combusted at the cessation of the experiment showed that volatilization of the ^{14}C-labeled trifluralin had occurred in the microcosm. The amounts of volatilization were less than 4% of the added radioactivity but did account for a greater loss of the herbicide than did mineralization. Laboratory experiments have shown that trifluralin volatized between 5% and 50% within the first 36 hours after application but there was little additional loss thereafter (Helling, 1976).

The results of this study show that more than 93% of the applied radioactivity was unaccounted for at the end of the experiment. Therefore, under the conditions of this

experiment, the trifluralin residues remaining in the soil environment may have the potential to cause injury to cereal crops grown in the rotation.

6.7 Summary and Conclusions

The mineralization and volatilization of ^{14}C-trifluralin accounted for a very small portion of the total applied radioactivity. The laboratory conditions in this study did not parallel environmental conditions in actual fields. Although optimal moisture and temperature in the microcosms may have existed to facilitate microbial growth, the conditions within the microcosm jars are not identical to field conditions, and may have contributed to the low amounts of trifluralin mineralization in soil. The amount of the herbicide remaining in the soils under the conditions of this experiment may cause injury to cereal crops that follow in the rotation.

CHAPTER 7

Sorption and Desorption Of Glyphosate from a Field Soil

7.1 Abstract

The herbicide glyphosate controls a very broad spectrum of weeds and has become an integral component within conservation-tillage and glyphosate tolerant cropping systems, making it currently the world's leading agrochemical. The purpose of this study was to determine whether phosphate fertilizer additions, potato crop rotations and the type of crops grown in rotation impacts the sorption and desorption behavior of glyphosate in the soil environment. Using batch equilibrium procedures, this study examined glyphosate sorption and desorption in the soil environment. Results indicated that the farm management practices studied did not have a statistically significant effect on the amount of glyphosate sorbed or desorbed in soil. For all treatments, greater than 98% of the glyphosate applied to the soil was sorbed onto soil mineral particles, with soil organic matter having minimal effect on the amount of glyphosate retained in soil. Less than 1% of the sorbed glyphosate was desorbed from soil. The implications of this study suggest that changes in soil organic matter amount due to cropping systems will not influence glyphosate behaviour in soil. Although theoretically possible, phosphate did not compete with glyphosate for sorption sites in soil at rates as high as eight times the recommended fertilizer application.

7.2 Introduction

Glyphosate is a non-selective, post emergent, systemic herbicide, which moves from treated foliage into roots and kills the entire plant (Anonymous, 1999). Essentially all plants are susceptible to the herbicide except for plants genetically engineered for resistance. Glyphosate has become the world's leading agrochemical and still is the fastest growing, facilitating the production of low cost input, wholesome food (Baylis, 2000). In Manitoba the market share of Roundup-Ready canola has increased from 0.9% in 1996 to 41.3 percent in 2000 (Park, 2002). The rapid acceptance of glyphosate-tolerant crops is due to multiple factors including its effective broad-spectrum weed control, low cost and simplicity (Shaner, 2000). The rapid and expansive use of this herbicide warrants a close scrutiny of the environmental impact that may occur over the coming years.

Glyphosate is strongly bound to the soil constituents, which greatly reduces its soil residual activity and risks of crop injury (Torstenson, 1985). Soil constituents such as clay minerals and humic substances exert the strongest influence on the sorption behavior of glyphosate in the soil environment (Sprankle et al., 1975; Piccolo et al., 1996).

The strong sorption of the herbicide precludes it from moving from the target site. Data from several soil-leaching studies indicates that glyphosate is fairly immobile in soils and therefore has little propensity for leaching in most soils (Franz et al., 1997). Research has also shown that negligible amounts of glyphosate have been detected in surface and runoff waters (Rueppel et al., 1977; Roy et al., 1989). Edwards et al. (1980) showed that in most cases less than 1% of the glyphosate was detected in surface runoff

waters when applied in watersheds planted to corn and pasture crops. Larney et al., (1999) showed that herbicides sorbed onto soil particles might be transported off the target site via wind erosion.

Phosphate fertilizers and glyphosate are both sorbed onto the soil constituents through the phosphonic acid moiety and therefore they may compete with each other for soil sorption sites (de Jonge and de Jonge, 1999). Studies have shown that phosphate additions to the soil decrease the amount of the herbicide sorbed (Sprankle et al., 1975; de Jonge and de Jonge, 1999). Continuous application of P fertilizers for crop production may result in accumulation in the soil (McKenzie et al., 1992). Since both phosphate (P) fertilizers and glyphosate are typically applied to the same field year after year there may be long-term consequences to consider. A decrease in glyphosate sorption with time may result in increased crop injury as the seedling may be exposed to greater concentrations of the herbicide in the soil solution (Franz et al., 1997). Alternatively, phosphate fertilizers may become less sorbed by soil when glyphosate is applied, increasing the availability of the nutrient for plant uptake.

7.3 Objective of the Study

The objective of this study is to determine the impact of phosphate fertilizer rates, soil organic matter content and the type of crop and crop rotation on the sorption and desorption behaviour of glyphosate in the soil environment.

7.4 Materials and Methods

7.4.1 Site description

The location and type of plots sampled from the MCDC experimental rotation are specified in chapter 3.

7.4.2 Soil Sampling and Preparation

The soil samples for the rotation study were collected in September 2000 to examine the impact of soil organic matter and type of crop rotation on the sorption - desorption behavior of glyphosate in soil. Four random soil samples (0-10 cm) per plot were taken using a Dutch auger. This depth was chosen on the basis that, although the herbicide is foliar applied, it becomes translocated to the root systems of the target plant. As such, the portions not utilized by the plant may end up in soil.The samples from each plot were composited and placed in plastic bags. The samples were stored at 4^0C until they were utilized for the experiment at which time they were air-dried and sieved using a 2mm mesh sieve.

The soil samples for the phosphate study were collected in May 2001 from the periphery of the rotational plots at the MCDC site to ensure that P fertilizers and glyphosate had not been applied to the soil. A spade was used for sampling and the soil was collected at a depth of 10 cm and placed in plastic bags. The soils were kept at 4^0C until used for the experiment. The samples were then air-dried for 24 hours and sieved through a 2 mm mesh in preparation for the experiment.

7.4.3 Soil Organic Carbon Analysis

Soil samples (5 g) from each of the 18 treatments were subjected to analysis for organic-carbon content. Inorganic carbon was removed prior to organic carbon measurement by adding 10 ml of 6M HCl in distilled water to the soil and heating the slurry on a hot plate for 10 minutes (Tiessen et al., 1983). The samples were then rinsed with 240 ml of distilled water to remove the inorganic ions from the soil. Soil organic carbon was determined by dry combustion of 0.12 g of oven-dried soil with a Leco model CHN 600 C and N determinator (Nelson and Sommers, 1982).

7.4.4 ^{14}C-Glyphosate Solutions

Both herbicide solutions were prepared to simulate field application rates of 0.36 kg of active ingredient per hectare when added to the 5 g soil samples. The field application rate added to the soil samples was calculated by determining the amount of the label rate of the herbicide that would be in the top 10 cm of the field soil after application.

The herbicide stock solution for the rotation study was prepared by adding ^{14}C-glyphosate (sp. act. 2.4 mCi/mmol; Sigma Chemical Co. St. Louis, MO) and analytical grade glyphosate (99% purity; SUPELCO. Bellefonte, PA.) to 0.01 M $CaCl_2$ (anti-dispersing agent) solution. The concentration of the herbicide solution contained 25 Bq of ^{14}C-glyphosate and 1 ug of analytical grade glyphosate per ml.

The herbicide stock solution for the phosphate study was prepared by adding glyphosate - (phosphonomethyl-^{14}C) (sp. act. 2.4 mCi/mmol; Sigma Chemical Co. St. Louis, MO) and analytical grade glyphosate (99 % purity; SUPELCO. Bellefonte, PA.) to a 0.01 M $CaCl_2$ (anti-dispersing agent) solution. The concentration of the herbicide

solution contained 45 Bq of ^{14}C-glyphosate and 3 ug of analytical grade glyphosate per ml. The field application rate added to the soil samples was calculated by determining the amount of the label rate of the herbicide that would be in the top 10 cm of the field soil after application.

7.4.5 Phosphate Solutions

The phosphate solutions for the phosphate application study were prepared by adding analytical grade KH_2PO_4 (Sigma Chemical CO. St. Louis, MO) to deionized water. The phosphate rates in 10 ml of the solution were 15 kg/ha, 30 kg/ha (which corresponded to a typical field application rate), 60 kg/ha, 120 kg/ha, and 240 kg/ha when added to the 5 g soils samples

7.4.6 Sorption Procedures

Standard batch equilibrium measurements were conducted in duplicate to quantify the sorption and desorption behaviour of glyphosate in the soil samples from the various rotational plots. The herbicide solution (15 ml) was added to 5 g of soil in 50 ml Teflon centrifuge tubes.

Standard batch equilibrium measurements were also utilized for the phosphate study, for quantifying the sorption of glyphosate in soil containing with various rates of P. The phosphate solution (10 ml) was added to 5 g of soil in centrifuge tubes representing ½, 1, 2, 4 and 8 X the typical application rate of P. The tubes were rotated for 1 hour to achieve uniform sorption of the phosphate in soil. Subsequently, 5 ml of the ^{14}C-glyphosate

solution was then added to each of the centrifuge tubes for a total of 15 ml of phosphate and herbicide solution in each tube.

For both experiments, tubes were then placed in a rotary shaker for 24 hours in the dark at room temperature to reach equilibrium. Subsequently, the soil slurries were centrifuged at 10,000 RPM for 10 minutes and 1 ml of the supernatant was sub-sampled in duplicate to quantify the concentration of the herbicide remaining in solution. The supernatant (1 ml) was placed in a 15 ml scintillation vial and 10 ml of Scintisafe, 30 % (Fisher Scientific, Fairlawn, New Jersey) was added. The vials were allowed to sit in a darkened room for 24 hours and then the radioactivity was quantified by a Beckman LS 7500 scintillation counter (LSC). The amount of radioactivity detected was subtracted from the amount initially applied to give the amount sorbed by the soil constituents and calculated as a percentage of the original amount applied. The sorption distribution coefficient, Kd [ml g^{-1}] was calculated assuming linear partitioning (1/n = 1) according to the following equation:

$$Kd = Cs/Ce \qquad \textbf{Equation 7.1}$$

where: Cs = the concentration of the herbicide sorbed by the soil at equilibrium [mg ml^{-1}] and Ce = the concentration of the herbicide in solution at equilibrium [mg ml^{-1}]. Greater Kd values indicate greater herbicide sorption by the soil relative to the smaller Kd values.

For the desorption procedures, an additional 5 ml of the supernatant was then removed from the centrifuge tubes and 7 ml of 0.01 M of $CaCl_2$ solution was added to bring the solution phase back to 15 ml. The batch-equilibrium procedures outlined for the sorption experiment were repeated and the amount of ^{14}C-glyphosate desorbed was quantified by LSC. Amount desorbed from the samples was calculated by the following method. The

herbicide remaining in 8 ml of solution after the sorption experiment was recorded (ug/ml). The amount of the herbicide remaining in the 15 ml solution after the desorption procedure was recorded (ug/ml). The amount of herbicide in solution after the sorption experiment was subtracted from the amount quantified for the desorption procedure. All desorption values were given as a percentage of the original herbicide applied in solution to the soil samples. The remaining portion of trifluralin was assumed to be sorbed onto the soil constituents. The LSC results for both sorption and desorption measurements were corrected by subtracting the background radiation.

7.4.7 Statistical Analysis

The data generated in the sorption - desorption experiments were subjected to one-way analysis of variance (ANOVA) using Sigmastat 2.03 software (1995 Access Softtek Inc.) and JMP IN software (SAS Institute, 1996). The linear regression analysis was performed using the SigmaPlot 2000 software (1986-2000 SPSS Inc.). The mean comparisons analyzed were designed to account for the unequal replicates in the study and were separated according to the Fisher LSD method at alpha = 0.05.

7.5 Results

The results of the sorption and desorption experiment showed that glyphosate was strongly bound onto the soil constituents regardless of the amount of soil organic matter, and type of crop rotation (Table 7.1). The lowest sorption values belonged to the alfalfa-alfalfa-alfalfa rotations (99.20 %) while the greatest values occurred in the potato–wheat-potato and potato-canola (underseeded to alfalfa)-alfalfa rotations (99.57 %). Clearly,

there was not a statistically significant difference among the rotation plots (P = 0.212). Similarly, the mean differences among the Kd values were not significantly different (P = 0.055). Less than 1.4 % desorption occurred in all soil samples irrespective of soil organic matter amounts or type of crop rotation and the mean differences were statistically significant (P = 0.0001). However, considering the very low desorption rates of all samples, the results would not be important in an actual field situation. Soil organic carbon content was the lowest in the canola-wheat-potato rotation (2.89 %) and the highest in the alfalfa-potato-canola (underseeded to alfalfa) rotation (3.74 %) (Table 7.1). However, the differences among the treatment groups were not statistically significant (P = 0.858).

Table 7.1 Sorption and desorption of glyphosate by soil in relation to fourteen crop rotations. Kd [ml g^{-1}] was determined by batch equilibrium experiments.

Rotation	% Organic-Carbon	% Sorption	Kd	% Desorption
Alfalfa-alfalfa-alfalfa	2.97a*	99.20a** (+/- 0.282)	372a** (+/- 123)	0.70a** (+/- 0.141)
alfalfa-potato-canola (underseeded to alfalfa)	3.74a*	99.60a** (+/- 0.141)	738a** (+/- 164)	0.85a** (+/- 0.212)
Canola (underseeded to alfalfa)-alfalfa-alfalfa	3.63a*	99.50a** (+/- 0.000)	616a** (+/- 9.20)	0.15b** (+/- 0.071)
Canola-potato-canola	3.18a** (+/- 0.327)	99.48a*** (+/- 0.403)	937a*** (+/- 840)	0.67b*** (+/- 0.150)
Canola-wheat-potato	2.89a** (+/- 0.536)	99.35a*** (+/- 0.208)	509a*** (+/- 193)	0.53b*** (+/- 0.126)
Oats-wheat-oats	3.36a*	99.50a** +/- 0.283)	648a** (+/- 406)	0.85a** (+/- 0.071)
Potato-canola (underseeded to alfalfa)-alfalfa	3.66a*	99.85a** (+/- 0.071)	2810a** (+/- 1325)	0.70a** (+/- 0.141)
Potato-oats-wheat	3.13a*	99.45a** (+/- 0.354)	712a** (+/- 452)	0.25b** (+/- 0.212)
Potato-wheat-canola	3.27a** (+/- 0.542)	99.68a*** (+/- 0.171)	1180a*** (+/- 759)	0.73b*** (+/- 0.171)
Potato-wheat-potato	3.46a*	99.85a** (+/- 0.171)	1847a** (+/- 919)	0.90a** (+/- 0.283)
Wheat-canola-wheat	3.13a*	99.55a** (+/- 0.171)	688a** (+/- 330)	0.35b** (+/- 0.212)
Wheat-potato-canola	3.33a*	99.60a** (+/- 0.141)	780a** (+/- 260)	1.05a** (+/- 0.071)
Wheat-potato-oats	3.52a*	99.70a** (+/- 0.141)	1164a* * (+/- 471)	1.30a** (+/- 0.141)
Wheat-potato-wheat	3.04a** (+/- 0.239)	99.68a*** (+/- 0.171)	1108a*** (+/- 707)	0.68b*** (+/- 0.237)

*One replicate.

**Means of 2 replicates followed by standard deviation.

***Means of 4 replicates followed by standard deviation.

#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

Although showing a greater contrast, type of crop grown in the year of sampling also had no significant influence on glyphosate sorption in soil (P = 0.331) (Table 7.2). The plots cropped to potato exhibited the lowest sorption values (99.36%) while the plots cropped to oats had the highest value (99.71%). The Kd values were lowest for the potato crop (575) and the highest for the oats crop (1068). The mean differences in Kd among the treatment groups were not significantly different (P = 0.252). All field samples had a desorption rate of less than 1% except for the plot cropped to oats (1.28%) and the mean differences among the treatment groups were not significantly different (P = 0.157). The plots where the potato crops grew had the least amount of organic carbon (3.09%) while the alfalfa plots contained the highest amounts (3.68%) (Table7.2). The differences in soil organic carbon among the various types of crops grown were not statistically significant (P = 0.105).

Table 7.2 Sorption and desorption of glyphosate by soil in relation to crop grown. Kd [ml g^{-1}] was determined by batch equilibrium experiments.

Crop	% Organic-C	% Sorption	Kd	% Desorption
Potato	**3.09a*#** (+/- 0.35)	**99.36a*#** (+/- 0.26)	**575a (+/- 329)**	**0.69a (+/- 0.18)**
Wheat	**3.20a**** (+/- 0.28)	**99.44a**** (+/- 0.26)	**630a (+/- 286)**	**0.55a (+/- 0.25)**
Canola	**3.21a***** (+/- 0.28)	**99.67a***** (+/- 0.10)	**957a (+/- 261)**	**0.77a (+/- 0.20)**
Alfalfa	**3.68a***** +/- 0.06)	**99.50a***** (+/- 0.08)	**596a (+/- 86)**	**0.56a (+/- 0.42)**
Oats	**3.52a****** (+/- 0)	**99.71a****** (+/- 0)	**1068a (+/ 0)**	**1.28a (+/- 0)**

*Means of six replicates followed by standard deviation.
**Means of five replicates followed by standard deviation.
***Means of three replicates followed by standard deviation.
****Means of one replicate followed by standard deviation.
#Means in the same column followed by the same letter are not statistically significant at $P < 0.05$ (One-way ANOVA).

Glyphosate was strongly sorbed onto the soil samples that received phosphate applications (Table 7.3). The lowest amount of sorption occurred in the control samples (99.28 %) and the highest occurred in the samples treated with 60 kg/ha (99.85%). There was a significant difference among the different phosphate application rates ($P = 0.009$). The mean differences were significant between the control samples and all treatments except for the 30 kg/ha samples and the 30 kg/ha samples were not significantly different from the other phosphate treatments. The sorption values among treatments were generally small with no consistent pattern in terms of amount of P applied and glyphosate behavior. The Kd values were highest for the 120 kg/ac (10288) treatment and lowest for the control treatment (308). There were significant differences among the treatment groups ($P = 0.0276$) (Table 7.3).

Table 7.3 Glyphosate sorption in soil in relation to phosphate application. Kd [ml g^{-1}] was determined by batch equilibrium experiments.

Treatment	% Sorption	Kd
Control	**99.28a*#**	**308a (+/- 8142)**
	(+/- 0.37)	
15 kg/ha	**99.77b***	**1367a (+/- 701)**
	(+/- 0.14)	
30 kg/ha	**99.52ab*c**	**625a (+/- 43)**
	(+/- 0.03)	
60 kg/ha	**99.85bcd***	**2165a (+/- 761)**
	(+/- 0.05)	
120 kg/ha	**99.90bcd***	**10288b (+/- 8143)**
	(+/- 0.144)	
240 kg/ha	**99.75b***	**1409a (+/- 790)**
	(+/- 0.11)	

*Means of three replicates followed by standard deviation.
#Means in the same column followed by the same letter are not statistically significant at $P < 0.05$ (One-way ANOVA).

The relation between soil organic carbon content and Kd for glyphosate sorption for all soil samples for the rotation experiment had an $R^2 = 0.098$ (Figure 7.1). The very low value for the R^2 indicates that the organic matter in the soil did not greatly influence the sorption behaviour of glyphosate in soil.

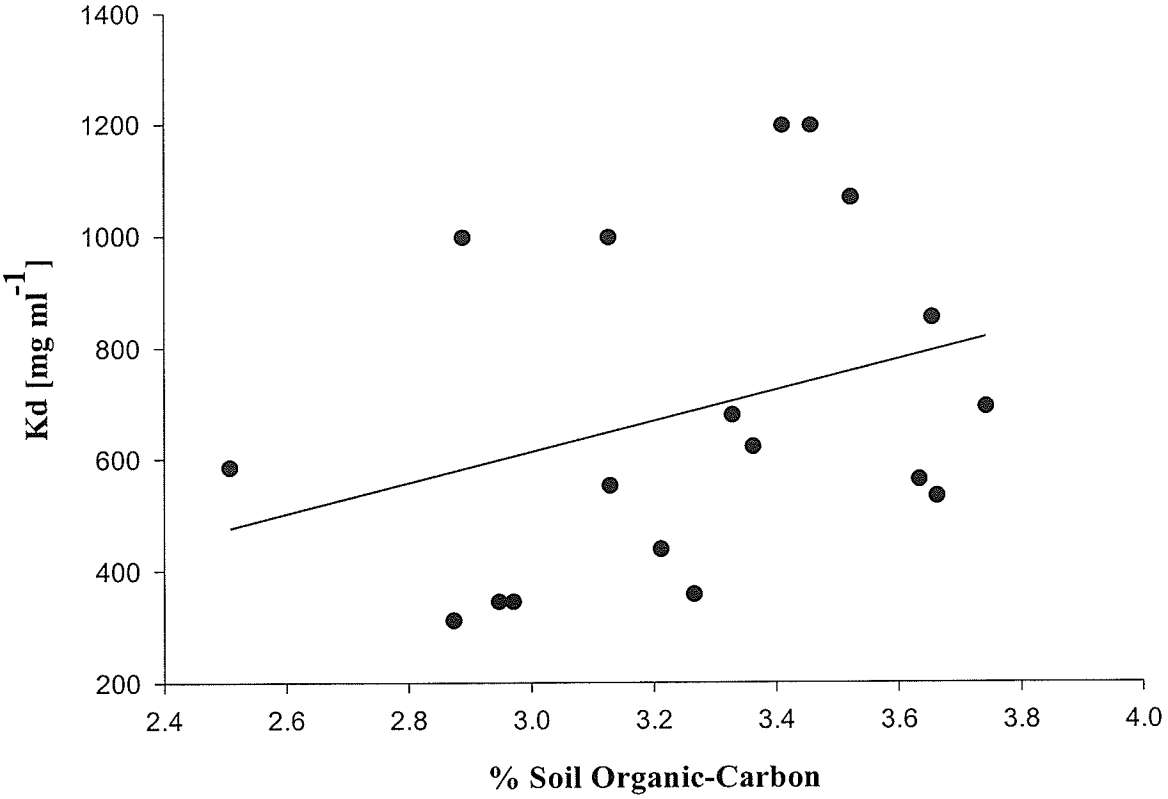

Figure 7.1 Relation between soil organic-carbon and glyphosate sorption by soil [Kd mg ml^{-1}]

7.6 Discussion

The results of the sorption and desorption experiment provide evidence that glyphosate is tightly bound to the soil mineral constituents and it's fate is not influenced by soil organic carbon content. The amounts desorbed were negligible as only two samples showed more than 1% desorption. This data confirms previous work that glyphosate is rapidly inactivated by sorption onto soil, thereby mitigating crop injury

(Franz et al., 1997). The data also shows that a very poor correlation exists between soil organic carbon and glyphosate sorption in the soil environment. Therefore the soil mineral environment may be more important for glyphosate sorption than the type of crop grown and resulting differential inputs of soil organic matter. These results are in sharp contrast with those of Piccolo et al. (1996) who claimed that humic substances exert a strong influence on glyphosate sorption in the soil. However, their sorption experiments were conducted solely on humic acids and not on actual field soil samples and this may account for the discrepancy in results.

Glyphosate was strongly sorbed onto all of the soil samples tested irrespective of the amount of phosphate added to the soil. An unexpected result to the experiment was that the soil samples that did not receive any phosphate additions had the lowest sorption rate. This contradicts the previous work that suggests high phosphate application rates decrease glyphosate sorption in soil (de Jonge and de Jonge, 1999). The results demonstrate that phosphate additions to the soil do not impact the sorption of glyphosate, suggesting that there are enough sorption sites in the soil samples to accommodate both the herbicide and fertilizer additions. Therefore, application of phosphate fertilizers and glyphosate in the same growing season would not likely result in crop injury by the herbicide or increased availability of the fertilizer to the crop. In contrast, research by de Jonge and de Jonge (1999) clearly indicated that phosphate additions to the soil presented strong competetion for glyphosate sorption, however their phosphate rates were highly unrealistic, approximately 540 ppmw or 50 times a recommended field application rate. A realistic field application rate of 10 ug of phosphate per gram of soil (10 ppmw) or 30 kg/ha, as used in this experiment better reflects actual farm management practices.

The strong sorption of glyphosate would prohibit leaching of the herbicide through the soil profile. Consequently, the Assiniboine Delta Aquifer, located at a depth of 1.7 to 5.2 meters below the MCDC site, and a vital source of drinking water for the surrounding community of the study site, is unlikely to be contaminated with the herbicide. Also, farm management factors such as the type of crop rotation and P fertilizer rates will not influence the risks of glyphosate leaching through the soil profile.

The most likely movement of the herbicide from the application site would be from wind erosion. In the absence of conservation farming practices, wind erosion remains one of the major forms of soil degradation on the Canadian Prairies (Wall et al., 1995). Estimates on inherent risk of wind erosion of bare soil in the Prairie Provinces ranges from low as 31% of the cultivated land to high to severe on 36 % of the total agricultural land depending on tillage practices (conventional versus minimum tillage) and soil texture (sand versus clay) (Wall et al., 1995). As such, glyphosate applied on erosion prone soils has the potential to move off-site and contaminate the environment, particularly since this study demonstrates that glyphosate has a strong potential to remain in the surface soil layer. The results of this study warrant additional research on the effect of wind erosion on environmental contamination by pesticides.

7.7 Summary and Conclusions

Glyphosate binds strongly to the soil constituents regardless of the type of potato crop rotation, the type of crop grown in the rotation, or the amount of inorganic phosphate added to the soil. The amount of soil organic carbon has very little impact of glyphosate

sorption and therefore the soil mineral content appears to exert a more profound effect on sorption of the herbicide.

The strong sorption of glyphosate to the soil constituents would reduce the likelihood of crop injury, contributing to the ubiquitous use of the herbicide. The sorption of the herbicide would also mitigate its leaching from the target site into groundwater sources. However, the potential does exist for the herbicide to be transported off site via soil particles by wind erosion. The landscape at the MCDC study site is level and therefore it is unlikely water erosion would occur.

CHAPTER 8

Mineralization of Glyphosate from a Field Soil

8.1 Abstract

The herbicide glyphosate controls a very broad spectrum of weeds and has become an integral component of conservation-tillage and glyphosate tolerant cropping systems, currently making it the world's leading agrochemical. The purpose of this study was to determine whether type of crop rotation and type of crop grown has an impact on the mineralization rates of glyphosate in soil. Soil microcosm experiments were set up to quantify the amount of glyphosate mineralized. The results of this study indicate that the type of crop rotation and crop grown had a significant effect on total glyphosate mineralization. The half-life of glyphosate in soil was less than 15 days in all samples and was not significantly different according to type of crop rotation and crop grown.

8.2 Introduction

Glyphosate (N- (phosphonomethyl) glycine) is the active ingredient in several herbicide formulations and is a non-selective, post emergent herbicide used in a variety of agricultural, industrial and domestic situations (Baylis, 2000).

Glyphosate degradation in soil occurs primarily through microbial activity (Sprankle et al., 1975; Moshier and Penner, 1978). It has been found that herbicide mineralization (the transformation of the herbicide into CO_2 and inorganic compounds) follows the kinetics of a first-order reaction (Eberbach, 1998). The rates of glyphosate degradation can vary considerably between different soil types, particularly among those with different textural classes and soil organic matter content (Nomura and Hilton, 1977; Mossier and Penner, 1978; Smith and Aubin, 1993). Degradation rates were lowest in fine textured soils containing the highest organic matter content. Humic acid extracts were found to sorb glyphosate more than clay minerals (Piccolo et al., 1996). This may explain the decrease in mineralization of the herbicide in soil with high organic matter content (Nomura and Hilton, 1977). Laboratory experiments concerning the degradation of ^{14}C-glyphosate in agricultural and forest soils have shown the half-life of the herbicide to range from 3 days to 22.8 years depending on soil microbial population and to the extent of soil binding onto the soil organic and mineral fractions (Rueppel et al, 1997; Franz et al., 1997).

Glyphosate degradation is reported to occur without a lag phase and appears to be a co-metabolic process under both aerobic and anaerobic conditions, and is influenced by the nature of the microbial population in the soil environment (Sprankle et al., 1975; Reuppel et al., 1977; Nomura and Hilton, 1977). A number of soil fungi and bacteria have been isolated that have the ability to degrade glyphosate in terrestrial environments (Franz et al., 1997; Klimek et al., 2001). The primary metabolic product is aminomethylphosphonic acid (AMPA) ultimately degrading to water, carbon dioxide and phosphate (Klimek et al., 2001).

The rhizosphere is the region in the soil under the immediate influence of plant roots. Analysis of the organic materials exuded by the plant roots revealed a wide assortment of aliphatic, amino, and aromatic acids, sugars and amino sugars which provided a nutritious environment for soil microbial growth and activity (Paul and Clark, 1996; Kandeler et al., 2002; Piutti et al., 2002). Indeed, studies have shown that herbicide degradation is more efficient in the rhizosphere than in bulk soils (Piutti, et al., 2002). Some of the bacterial species capable of degrading glyphosate in the soil environment are *Pseudomonas*, *Rhizobium* and *Arthrobacter* (Jacob et al., 1991; Pipke et al., 1987; Liu et al., 1991).

8.3 Objective of the Study

The objectives of this study were to determine whether short-term changes in crop rotation or the type of crop grown had an impact on glyphosate mineralization in the soil environment.

8.4 Materials and Methods

8.4.1 Site Description

The soil samples obtained from the MCDC experimental plots are specified in Chapter 3.

8.4.2 Soil Sampling and Preparation

The soil samples for this study were collected in September 2000. A Dutch auger was used to obtain four random samples per plot from the 0 to 10 cm depth in the soil. The samples from each plot were composited and placed in plastic bags. After each plot was sampled the auger was rinsed in a bleach solution to prevent microbial contamination between plots. The samples were stored at 4°C freezer until they were utilized for the experiment.

8.4.4 Soil Organic Carbon Analysis

Soil samples (5 g) from each plot were subjected to organic-carbon analysis. Inorganic carbon was removed prior to organic carbon measurement by adding10 ml of 6M HCl in distilled water to the soil and heating the soil slurry on a hot plate for 10 minutes (Tiessen et al., 1983). The samples were rinsed with 240 ml of distilled water to remove the inorganic ions present. Soil organic carbon was determined by dry combustion of 0.12 g of oven-dried soil with a Leco model CHN 600 C and N determinator (Nelson and Sommers, 1982).

8.4.5 Microcosm Apparatus

The microcosm jars used in this experiment consisted of 1-litre Mason jars with screw top lids. A 50 ml beaker containing 25 g of field-moist soil was placed in the jar. Sufficient water was added to the soil samples to reach field capacity. A 15 ml scintillation vial containing 5 ml of 1 M NaOH solution was added to the jars to trap the

$^{14}CO_2$ evolved due to herbicide mineralization by soil microorganisms. Prior to glyphosate application, the jars were pre-incubated at 20°C on the dark for two weeks to increase the metabolic activity of the soil microorganisms. At day 35, 1 ml of water was added to the soil samples restoring them to field capacity.

For this study, a 1 ml portion of a solution containing 0.65 uCi of ^{14}C-methyl-labeled glyphosate (Sigma Chemical Co., St. Louis MO) was added drop wise by pipette to each soil. This corresponded to disintegrations per minute (DPM) count of 1.47 x 10^6 per ml. The amount of active ingredient associated with this application is equivalent to 3 times the recommended field application rate of 0.890 kg/ha of active ingredient.

8.4.6 Monitoring Glyphosate Mineralization

The 15 ml scintillation vial containing 1 M NaOH was used to trap any $^{14}CO_2$ evolved during the course of the experiment. For each of the soil samples the sealed jars created a closed environment which allowed for the containment and trapping of the mineralized ($^{14}CO_2$) portion of the radio labeled compound. For the rotation study the vials were in place for 168 days and were changed at regular intervals. Initially, the vials were replaced on day 2, 5, and 8 following herbicide application as the initial microbial activity and glyphosate degradation proceeded more rapidly at this time than later in the experiment. The vials were then changed every week for two months and then every two weeks until the cessation of the experiment.

After the $^{14}CO_2$ traps were removed from the microcosms, 8 ml of Scintisafe was added directly to each vial. The vials were allowed to sit in the dark at room temperature for 24 hours before the radioactivity was quantified by a Beckman LS 7500 scintillation

counter. The level of radioactivity per trap was given as disintegrations per minute (DPM). These values were then used to calculate the amount of $^{14}CO_2$ that had evolved during the trap's placement in the soil microcosm. The amount of $^{14}CO_2$ evolved was calculated as a percentage of the original amount of ^{14}C-glyphosate applied.

8.4.7 Mathematical and Statistical Analysis

The mineralization activity of the ^{14}C-glyphosate was conducted by measuring the ^{14}C that evolved as carbon dioxide and calculating it as a percent of the radioactivity that was initially added to the soil samples. A first order curve fitting was performed using SigmaPlot 2000 software (1986-2000 SPSS Inc.) and the following equation.

$$A_t = A_F (1\text{-}e^{-KF\,t}) \qquad \textbf{Equation 8.1}$$

Where A_t = percent degradation at time t (days), A_F = percent of added ^{14}C that has evolved at time infinity for the first order curve, and K_F = the degradation rate constant (days $^{-1}$) for the first order curve.

The K values that were determined by curve fitting were then used to calculate the half-life for glyphosate in each of the sampled plots. The half-life is calculated based on the percent of the chemical that was mineralized, therefore the half-life presented here is an indication of the time it takes for 50% of the mineralizable fraction to be mineralized. The following equation was used:

$$T^{1/2} = \ln 2/K \qquad \textbf{Equation 8.2}$$

JMP IN software (1996 SAS Institute Inc.) was used to perform a one-way analysis of variance (ANOVA) on the cumulative degradation at 21 days, which is when the most rapid mineralization occurred, day 105, which is the approximate length of the Manitoba

field crop growing season, and 168 days, the cessation of the experiment. A one-way analysis of variance (ANOVA) was also used to quantify the impact of crop rotation and type of crop grown on glyphosate half-lives in soil. The mean comparisons analyzed were designed to account for the unequal replicates in the study and were separated according to the Fisher LSD method at alpha = 0.05.

8.5 Results

The mineralization of glyphosate proceeded without a lag phase in all soil samples (Figure 8.1). After approximately 7 weeks the rate of glyphosate metabolism began to noticeably slow down in all of the soils. At this time $^{14}CO_2$ production had reached approximately 8% of total mineralization.

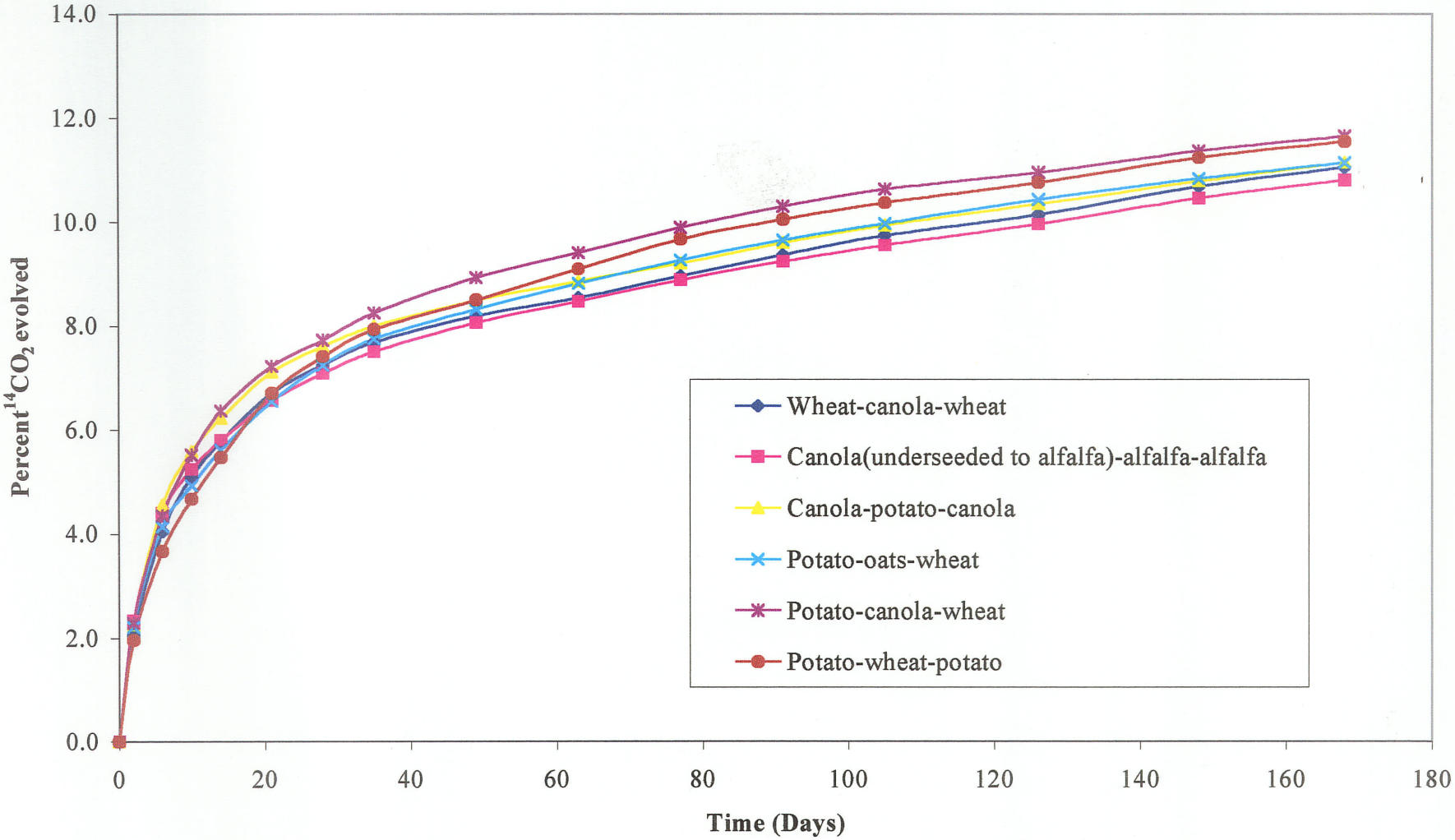

Figure 8.1 Glyphosate mineralization in soils with six different crop rotations. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$

After 21 days of incubation the mineralization amounts of glyphosate ranged from 6.33% in the wheat-potato-oats rotation to 7.69% in the oats-wheat-oats rotations (Table 8.1). The amounts of mineralization between the different crop rotations were significantly different (P = 0.007). (Table 8.1) The canola (underseeded to alfalfa)-alfalfa-alfalfa rotation was significantly lower than the wheat-potato-canola, oats- wheat-oats and canola-wheat-potato rotations.

Table 8.1 Mineralization of glyphosate in soil from fourteen crop rotations after 21 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop Rotation	$^{14}CO_2$ Evolved (%)
Alfalfa-alfalfa-alfalfa	7.06a*# (+/-0.200)
alfalfa-potato-canola (underseeded to alfalfa)	7.02a* (+/- 0.910)
Canola (underseeded to alfalfa)-alfalfa-alfalfa	5.54ab* (+/- 0.273)
Canola-potato-canola	7.11a** (+/- 0.482)
Canola-wheat-potato	7.53ae** (+/- 0.305)
Oats-wheat-oats	7.69ad* (+/- 0.101)
Potato-canola (underseeded to alfalfa)-alfalfa	6.65a* (+/- 0.643)
Potato-oats-wheat	6.77a* (+/- 0.428)
Potato-wheat-canola	6.58b** (+/- 0.967)
Potato-wheat-potato	6.50f* (+/- 0.214)
Wheat-canola-wheat	6.00d* (+/- 0.663)
Wheat-potato-canola	7.00ac* (+/- 0.489)
Wheat-potato-oats	6.33e* (+/- 1.318)
Wheat-potato-wheat	6.76a** (+/- 0.910)

*Means of 3 replicates followed by standard deviation.
**Means of 6 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

After 105 days of incubation the mineralization of glyphosate ranged from 8.35% in the canola(alfalfa)-alfalfa-alfalfa rotations to 11.2% in the canola-wheat-potato rotations (Table 8.2). The amounts of mineralization between the different crop rotations were significantly different (P = 0.0001). (Table 8.2). The canola (underseeded to alfalfa)-alfalfa-alfalfa rotation was significantly lower than the wheat-potato-canola, oats- wheat-oats, canola-wheat-potato, potato-oats-wheat, and wheat-potato-wheat rotations.

Table 8.2 Mineralization of glyphosate in soil from six crop rotations after 105 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop Rotation	$^{14}CO_2$ Evolved (%)
Alfalfa-alfalfa-alfalfa	10.0a*# (+/-0.470)
alfalfa-potato-canola (underseeded to alfalfa)	10.2a* (+/- 0.356)
Canola (underseeded to alfalfa)-alfalfa-alfalfa	8.35ab* (+/- 0.148)
Canola-potato-canola	9.94 ac** (+/- 0.445)
Canola-wheat-potato	11.2ac** (+/- 0.591)
Oats-wheat-oats	11.0ac* (+/- 0.065)
Potato-canola (underseeded to alfalfa)-alfalfa	9.66a* (+/- 0.624)
Potato-oats-wheat	10.9a* (+/- 0.529)
Potato-wheat-canola	9.36a** (+/- 0.883)
Potato-wheat-potato	9.72a* (+/- 0.681)
Wheat-canola-wheat	9.13a* (+/- 0.578)
Wheat-potato-canola	10.9ac* (+/- 0.605)
Wheat-potato-oats	9.08a* (+/- 1.211)
Wheat-potato-wheat	10.4ac** (+/- 0.771)

*Means of 3 replicates followed by standard deviation.
**Means of 6 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

After 168 days of incubation the mineralization of ^{14}C-glyphosate as quantified by evolution of $^{14}CO_2$ was less than 12% in each of the fourteen crop rotations (Table 8.1). The greatest mineralization occurred in the canola-wheat-potato rotation (12.6%) and the least amount occurred in the canola (alfalfa)-alfalfa-alfalfa rotation (9.56%) (Table 8.3). The mineralization differences among the treatment groups were statistically significant (P = 0.0001). The canola (underseeded to alfalfa)-alfalfa-alfalfa rotation was significantly lower than the wheat-potato-canola, oats- wheat-oats, canola-wheat-potato, potato-oats-wheat, and wheat-potato-wheat, alfalfa-potato-canola (underseeded to alfalfa) and the alfalfa-alfalfa-alfalfa rotations.

Table 8.3 Mineralization of glyphosate in soil from six crop rotations after 168 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop Rotation	$^{14}CO_2$ Evolved (%)
Alfalfa-alfalfa-alfalfa	11.5b*# (+/-0.432)
Alfalfa-potato-canola (underseeded to alfalfa)	11.6c* (+/- 0.289)
Canola (underseeded to alfalfa)-alfalfa-alfalfa	9.56d* (+/- 0.292)
Canola-potato-canola	11.1e** (+/- 0.624)
Canola-wheat-potato	12.6a** (+/- 0.476)
Oats-wheat-oats	11.9a* (+/- 0.089)
Potato-canola (underseeded to alfalfa)-alfalfa	10.6f* (+/- 0.690)
Potato-oats-wheat	12.0a* (+/- 0.067)
Potato-wheat-canola	10.3g** (+/- 0.750)
Potato-wheat-potato	10.9h* (+/- 0.905)
Wheat-canola-wheat	10.5i* (+/- 0.711)
Wheat-potato-canola	11.8a* (+/- 0.660)
Wheat-potato-oats	9.95j* (+/- 1.39)
Wheat-potato-wheat	11.7a** (+/- 0.675)

*Means of 3 replicates followed by standard deviation.
**Means of 6 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at P < 0.05 (One-way ANOVA

The mineralization of glyphosate after 21 days ranged from 6.33 % in the oat plots to 7.30% in the potato plots (Table 8.4). Glyphosate mineralization was significantly greater in potato cropped soils, relative to soils cropped to wheat, canola, alfalfa and oats (P = 0.015).

Table 8.4 Mineralization of glyphosate in soil from five crops after 21 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop	$^{14}CO_2$ Evolved (%)
Potato	**7.30a*# (+/- 0.470)**
Wheat	**6.56abc** (+/- 0.701)**
Canola	**7.01ab*** (+/- 0.950)**
Alfalfa	**6.41abc*** (+/- 0.879)**
Oats	**6.33ab**** (+/- 1.318)**

*Means of 18 replicates followed by standard deviation.
**Means of 15 replicates followed by standard deviation.
***Means of 9 replicates followed by standard deviation.
****Means of 3 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

The mineralization of glyphosate after 105 days in relation to crop grown ranged from 9.08% where oats was grown to 10.51% where potato was grown (Table 8.5). Again, soils cropped to potatoes showed significantly greater glyphosate mineralization, relative to soils cropped to alfalfa and oats (P = 0.021). Soils cropped to wheat, canola, alfalfa and oats showed similar glyphosate mineralization rates.

Table 8.5 Mineralization of glyphosate in soil from five crops after 105 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop	$^{14}CO_2$ Evolved (%)
Potato	**10.51a* (+/- 0.777)**
Wheat	**9.84a** (+/- 0.872)**
Canola	**9.98a*** (+/- 1.050)**
Alfalfa	**9.42a*** (+/- 0.912)**
Oats	**9.08a**** (+/- 1.211)**

*Means of 18 replicates followed by standard deviation.
**Means of 15 replicates followed by standard deviation.
***Means of 9 replicates followed by standard deviation.
****Means of 3 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

The mineralization of glyphosate after 168 days in relation to crop grown ranged from 9.95% in the oat plots and 11.81% in the plots where potato crops were grown (Table 8.6). There was a significant difference among the treatment groups (P = 0.004). Plots where potatoes were grown showed significantly greater glyphosate mineralization relative to plots with canola, alfalfa and oats. Soils with wheat showed significantly greater mineralization amounts relative to soils cropped to oats.

Table 8.6 Mineralization of glyphosate in soil from five crops after 168 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Crop	$^{14}CO_2$ Evolved (%)
Potato	**11.81a*# (+/- 0.791)**
Wheat	**11.28ac** (+/- 0.898)**
Canola	**10.91bc*** (+/- 1.046)**
Alfalfa	**10.58bc*** (+/- 0.988)**
Oats	**9.95b**** (+/- 1.387)**

*Means of 18 replicates followed by standard deviation.
**Means of 15 replicates followed by standard deviation.
***Means of 9 replicates followed by standard deviation.
****Means of 3 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

The half-life of glyphosate in soil was the shortest in the canola-potato-canola rotation (9.7 days) and the longest in the potato-oats-wheat rotation (14.9 days) (Table 8.7). There was not a significant difference in the half-life of glyphosate in the soil among the different crop rotations (P = 0.501).

Table 8.7 Half-life of glyphosate in soil from fourteen crop rotations (Days).

Crop Rotation	Half-life (Days)
Alfalfa-alfalfa-alfalfa	**10.5a*# (+/- 0.0)**
Alfalfa-potato-canola (underseeded to alfalfa)	**11.1a* (+/- 0.0)**
Canola (underseeded to alfalfa)-alfalfa-alfalfa	**10.9a* (+/- 0.0)**
Canola-potato-canola	**9.70a** (+/- 1.7)**
Canola-wheat-potato	**12.2a** (+/- 2.7)**
Oats-wheat-oats	**9.50a* (+/- 1.4)**
Potato-canola (underseeded to alfalfa)-alfalfa	**10.2a* (+/- 0.0)**
Potato-oats-wheat	**14.9a* (+/- 0.0)**
Potato-wheat-canola	**10.9a** (+/- 0.0)**
Potato-wheat-potato	**14.1a* (+/- 0.0)**
Wheat-canola-wheat	**10.9a* (+/- 0.0)**
Wheat-potato-canola	**12.9a* (+/- 0.0)**
Wheat-potato-oats	**9.71a* (+/- 0.0)**
Wheat-potato-wheat	**13.5a** (+/- 2.2)**

*Means of 1 replicate followed by standard deviation.
**Means of 2 replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at P < 0.05 (One-way ANOVA

The half-life of glyphosate in the soil was the shortest in the canola crop (10.5 days) and the longest in the wheat crop (13.1 days) (Table 8.8). There was not a significant difference in the half-life of the herbicide in the soil among the different crops (P = 0.278).

Table 8.8 Half-life of glyphosate in soil from five crops (Days)

Crop	Half-life (Days)
Oats	**10.6a*# (+/- 0)**
Canola	**10.5a** (+/- 0.72)**
Alfalfa	**10.6a** (+/- 0.47)**
Potato	**11.2a*** (+/- 2.35)**
Wheat	**13.1a**** (+/- 1.80)**

*Means of one replicates followed by standard deviation.
**Means of nine replicates followed by standard deviation.
***Means of eighteen replicates followed by standard deviation.
****Means of fifteen replicates followed by standard deviation.
#Means followed by the same latter are not significantly different at $P < 0.05$ (One-way ANOVA).

8.6 Discussion

During the 168-day incubation period of the soil samples from the rotation study there was a constant evolution of $^{14}CO_2$ samples, which is indicative of microbial activity. Mineralization of the herbicide occurred without a lag phase indicating that a microbial population capable of metabolizing the herbicide resided in the soil. The amount of mineralization that occurred after 168 days accounted for less than 12% of the applied radioactivity. The mineralization rate decreases rapidly after three weeks at which time greater than 6% of the applied radioactivity was mineralized. The slowdown in glyphosate mineralization after three weeks reflect the strong sorption in the soil matrix and hence reduced availability for decomposition (Eberbach, 1998). For most pesticides, decomposition can only occur from the labile phase (Eberbach, 1998). Reuppel et al. (1977) contend that this is particularly true for glyphosate, as in their experiment, degradation had noticeably slowed down after seven days reflecting strong sorption of the substrate and reduced availability for decomposition.

The soil microcosms are considered a relatively closed biological system and this has implications for the dynamics of microbial metabolism and growth (Tate, 1995). As a consequence, nutrient supplies are often limited and metabolic waste products accumulate. In an open biological system, microbial cells are exposed to fresh supplies of growth nutrients and wastes do not necessarily accumulate in the vicinity of the colony or individual cell (Tate, 1995). The size of bacterial colonies that are able to degrade a

herbicide may be reduced in a closed system versus an open one and this must be kept in mind when proceeding with data analysis.

The mineralization of glyphosate after 21, 105 and 168 days were significantly different among the different crop rotations in the 2000 growing season. The mineralization amounts in the rotations ranged from 9.6% in the canola (underseeded to alfalfa)-alfalfa-alfalfa rotations to 12.6% in the canola-wheat-potato rotations. The low mineralization amounts indicated that most of the pesticide remained in soil. The glyphosate remaining in the soil throughout the growing season would not likely injure crops, as the herbicide activity in the soil is very low (Grossard and Atkinson, 1985).

The half-life of glyphosate mineralization in the soil environment was less than 15 days in all of the plots. Other laboratory studies have shown that the half-life of glyphosate in soil ranged from 3 days to 23 years depending on the soils physical and chemical properties (Nomura and Hilton, 1976; Franz et al, 1997)).

The mineralization and therefore the half-life of glyphosate in the soils from the MCDC plots had similar values regardless of the treatment. This may be attributed to the uniform soil and environmental conditions existing at the experimental site. Studies have shown that herbicide mineralization is influenced by the soil microbial population, organic matter content, soil mineralogy and the extent of soil binding (Sprankle et al., 1975; Rueppel et al., 1977; Nomura and Hilton, 1977; Mossier and Penner, 1978) The crop rotation study was in its third year and therefore the soil physical and chemical properties may not have been modified to an extent sufficient to impact on glyphosate behavior.

8.7 Summary and Conclusions

The results of the soil microcosm studies have shown glyphosate undergoes microbial degradation in the soil environment. The mineralization amounts were significantly different among the crop rotations and among the type of crop grown. The half-life of the herbicide was less than 15 days for all experimental plots. Despite the fact that approximately 90% of the herbicide remained in the soil after 168 days, the strong binding would likely inactivate the herbicide, mitigating the possibility of crop injury.

CHAPTER 9

Mineralization and Sorption of Glyphosate in Soils Amended with Humic Acid and Nitrogen and Phosphate Fertilizer

9.1 Abstract

The purpose of this study was to determine whether soils amended with humic acid (HA) or nitrogen (N) and phosphorus (P) fertilizers had an impact on the soil sorption of glyphosate and consequently, the mineralization rate of the herbicide in the soil environment. The experiments were conducted in order to assess what may occur in a long-term crop rotation (i.e., 10 years). Batch equilibrium procedures were utilized to determine the sorption of glyphosate in soil and soil microcosms were utilized to quantify the mineralization of the herbicide in the soil. The sorption of glyphosate was very high in all treatments (> 95%) and only the soils amended with 10% HA showed a significant difference. Significant mineralization amounts occurred in soils amended with HA and high rates of N and P fertilizers. The half-life of glyphosate ranged from 2.5 days in the soils amended with 10% HA to 13.3 days in the soils amended with 40 kg/ha of P fertilizer. This study has shown that soil sorption of glyphosate is not directly linked to the mineralization of the herbicide in soil.

9.2 Introduction

The physical and chemical properties of the soil as well as the chemical input from agricultural practices may impact the sorption and mineralization of glyphosate in soil

(Sprankle et al., 1975; Nomura and Hilton, 1977; Michel and Mew, 1998; de Jonge et al., 2001). Nitrogen fertilizers applied to high pH soils will be converted to ammonium, which may be toxic to plants and soil microorganisms (Havlin et al., 1999). The effect of urea additions to the soil at a rate of 200 kg/ha reduced the population of *Ralstonia solanacearum* (Michel and Mew, 1998). The decrease in the bacterial species was attributed to the toxic effects of high soil pH during urea hydrolysis and to the soil nitrite accumulation. As the microbial population in the soil decreases, the rate of herbicide mineralization would likely be reduced (Parr and Smith, 1971).

Phosphate fertilizers and glyphosate are both sorbed onto the soil constituents through the phosphonic acid moiety and therefore may compete with each other for soil sorption sites (de Jonge and de Jonge, 1999). Since the sorption mechanisms are similar for the two compounds, the long term increase of the phosphorus status in agricultural soils may lead to reduced sorptive strength of glyphosate (de Jonge et al., 2001). The reduced sorption may increase the availability of the herbicide for mineralization by the soil microbes (Moshier and Penner, 1978)

Piccolo et al. (1996) found that humic acids adsorbed glyphosate more than clay minerals and occurs through multiple hydrogen bonds. The degree of binding strength of glyphosate with the soil constituents will determine the rate of the herbicide mineralization in the soil (Nomura and Hilton, 1977; Moshier and Penner, 1978).

9.3 Objective of the Study

The objectives of this study were to determine whether soils amended with humic acid and nitrogen and phosphorous fertilizers had an impact on the sorption and mineralization of glyphosate in soil.

9.4 Materials and Methods

9.4.1 Site Description

The field site for this study is outlined in Chapter 3.

9.4.2 Soil Sampling and Preparation

The soil samples for the fertilizer and humic acid study were collected in May 2000 from the periphery of the rotational plots at the MCDC site to ensure that fertilizers and glyphosate had not been applied to the soil. A spade was used for sampling and the soil was collected to a depth of 10 cm and placed in plastic bags. The soils were kept at 4^0C until utilized for the experiment.

9.4.3 Fertilizer Analysis

Soil sample analyses for the fertilizer application experiment were conducted by NORWEST LABS (Winnipeg, MB). The nitrate-N from the soil samples were determined by potassium chloride extraction method (McKeague, 1978) and the available

phosphorus was determined by the "modified Kelowna" extraction method (Ashworth and Mrazek, 1995).

9.4.4 Soil Organic Carbon Analysis

Soil samples (5 g) for the control and humic acid amended soils from were subjected to organic-carbon analysis at the beginning (0 days) and the end (14 days) of the preliminary soil incubation period. Inorganic carbon was removed prior to organic carbon measurement by adding10 ml of 6M HCl in distilled water to the soil and heating the soil slurry on a hot plate for 10 minutes (Tiessen et al., 1983). The samples were rinsed with 240 ml of distilled water to remove the inorganic ions present. Soil organic carbon was determined by dry combustion of 0.12 g of oven-dried soil with a Leco model CHN 600 C and N determinator (Nelson and Sommers, 1982).

9.4.5 Nitrogen and Phosphorous Amendments

The nitrogen fertilizer solution for the study was prepared by adding analytical grade $(NH_2)_2CO$, (urea), (MALLINCKRODT Specialty Chemicals, Paris, KY) to deionized water. The urea fertilizer had a carbon:nitrogen ratio of 0.25:1.The N fertilizer rates in the solution corresponded to 30 kg/ha and 300 kg/ha when added to the 25 g soil samples. The rates corresponded to 38 and 380 ug of N per g of soil. Typical application rates in Manitoba range from 60 –150 kg/ha depending on the type of crop grown.

The phosphorus fertilizer solutions for the study were prepared by adding analytical grade $KHPO_4$ (Sigma Chemical CO. St. Louis, MO.) to deionized water. The P fertilizer rates in the solution corresponded to 40 kg/ha and 400 kg/ha when added to the 25 g soil

samples. The rates corresponded to 18 and 180 ug of P per g of soil. Typical application rates in Manitoba range from 30 – 70 kg/ha depending on the type of crop grown.

After the fertilizers were added to the soil samples, the microcosms were incubated for two weeks and the samples were tested for fertilizer amounts at the beginning and the end of the incubation period.

9.4.6 Humic Acid Amendments

Humic acid (Fluka Chemika, Sigma Chemical CO. St. Louis, MO) was added to the soil samples to represent 2% and 10% of the soil content. The carbon:nitrogen ratio for the HA was 64:1 (Picton, 2003). Soil samples (5 g) for the control and humic acid amended soils were subjected to organic-carbon analysis at the beginning (0 days) and the end (14 days) of the preliminary soil incubation period.

9.4.7 Microcosm Apparatus

The soil microcosm apparatus consisted of 1 – liter Mason jars with screw top lids. A 50 ml beaker containing 25 g of field-moist soil was placed in the jar. Sufficient water was added to the soil samples to reach field capacity. A 15 ml scintillation vial containing 5 ml of 1 M NaOH solution was added to the jars to trap the $^{14}CO_2$ evolved due to the herbicide mineralization by the soil microorganisms. Prior to glyphosate application, the jars were incubated at 20 °C in the dark for two weeks to increase the metabolic activity of the soil microorganisms. At day 70, 1 ml of water was added to the soil samples restoring them to field capacity.

The fertilizer and humic acid (HA) treatments were prepared in triplicate as follows: (1) 2% humic acid, (2) 10% humic acid, (3) 40 kg/ha N, (4) 400 kg/ha N, (5) 30 kg/ha P, (6) 300 kg/ha P, and (7) the control (no humic acid or fertilizer additions).

A 1 ml portion of the herbicide solution containing ^{14}C-methyl-labeled glyphosate and analytical grade glyphosate (99 % purity; SUPELCO. Bellefonte, PA.), in deionized water was mixed into the soil samples. The concentration of the solution was 0.17uCi of ^{14}C-glyphosate and 0.7 ug of analytical grade glyphosate per ml, which is the equivalent of a recommended field application rate of 0.890 kg/ha of active ingredient, which is a typical field rate for MB field crops. This corresponds to disintegrations per minute (DPM) count of 3.75×10^5 per ml of herbicide solution.

9.4.8 Monitoring Glyphosate Mineralization

The 15 ml scintillation vial containing 1 M NaOH was used to trap any $^{14}CO_2$ evolved during the course of the experiment. For each of the soil samples, the sealed jars created a closed environment which allowed for the containment and trapping of the mineralized $^{14}CO_2$ portion of the radio labeled compound. Initially, the traps were changed every four days following herbicide application as the microbial activity and degradation proceeded more rapidly at this time during the first four weeks and then every two weeks thereafter. The experiment was conducted over a 104-day period.

After the $^{14}CO_2$ traps were removed from the microcosms, 8 ml of Scintisafe was added directly to each vial. The vials were allowed to sit in the dark at room temperature for 24 hours before the radioactivity was quantified by a Beckman LS 7500 scintillation counter. The level of radioactivity per trap was given as disintegrations per minute

(DPM). These values were then used to calculate the amount of $^{14}CO_2$ that had evolved during the trap's placement in the soil microcosm. The amount of $^{14}CO_2$ evolved was calculated as a percentage of the original amount of ^{14}C-glyphosate applied.

9.4.9 Sorption Procedures

The herbicide stock solution was prepared by adding ^{14}C-glyphosate (sp. act. 2.4 mCi/mmol; Sigma chemical co. St. Louis, MO) and analytical grade glyphosate (99% purity; SUPELCO. Bellefonte, PA.) to 0.01 M $CaCl_2$ (anti-dispersing agent) solution. The concentration of the ^{14}C-glyphosate solutions was 18.3 Bq per ml for the control and humic acid samples and 18.4 Bq for the samples amended with N and P fertilizers and contained 1 ug per ml of analytical grade glyphosate.

The nitrogen fertilizer solutions for the study were prepared by adding analytical grade $(NH_2)_2CO$ (MALLINCKRODT Specialty chemicals, Paris, KY) to 0.01 M $CaCl_2$ solution. The N rates corresponded to 30 kg/ha and 300 kg/ha when added to the 5 g samples. The rates corresponded to 38 and 380 ug of N per g of soil. The rates were consistent with those used in the mineralization study.

The phosphorus fertilizer solutions were prepared by adding analytical grade $KHPO_4$ (Sigma Chemical CO. St. Louis, MO.) to 0.01 M $CaCl_2$ solution. The P fertilizer rates in the solution corresponded to 40 kg/ha and 400 kg/ha when added to the 5 g soil samples. The rates corresponded to 18 and 180 ug of P per g of soil. The rates were consistent with those used in the mineralization study.

The 5 g soil samples for the sorption experiment were amended to contain 2% and 10% humic acid, corresponding to the rates utilized for the mineralization experiment.

Standard batch equilibrium measurements were conducted in triplicate for all of the treatments. The phosphate solution (10 ml) was added to 5 g of soil in Teflon centrifuge tubes representing 30 and 300 kg/ha. The tubes were rotated for 1 hour to achieve uniform sorption of the phosphate by soil. Subsequently, 5 ml of ^{14}C-glyphosate was added to the tubes for a total of 15 ml of phosphate and herbicide solution. The nitrogen solution (10 ml) was added to the soil and then 5 ml of ^{14}C-glyphosate was added for a total of 15 ml of nitrogen and herbicide solution. The tubes were not rotated for 1 hour prior to addition of the glyphosate solution as the sorption of the nitrogen is not expected to occur as rapidly as the phosphate in solution (Flaten, 2002). For the humic acid treatments, the ^{14}C-glyphosate solution (15 ml) was added to the soil samples amended with 2% and 10% humic acid.

For all treatments the tubes were then placed in a rotary shaker for 24 hours in the dark at room temperature to reach equilibrium. Subsequently the soil slurries were centrifuged at 10,000 RPM for 10 minutes and 1 ml of the supernatant was sub-sampled in duplicate to quantify the concentration of the herbicide remaining in solution. The supernatant (1ml) was placed in a 15 ml scintillation vial and 10 ml of Scintisafe, 30% (Fisher Scientific, Fairlawn, New Jersey) was added. The vials were allowed to sit in a darkened room for 24 hours and then the radioactivity was quantified by a Beckman LS 7500 scintillation counter. The amount of radioactivity detected was subtracted from the amount initially applied to give the amount sorbed by the soil constituents. The sorption distribution coefficient, Kd [ml g-1] was calculated assuming linear partitioning (1/n = 1) according to the following equation:

$$Kd = Cs/Ce \qquad \textbf{Equation 9.1}$$

where: Cs = the concentration of the herbicide sorbed by the soil at equilibrium [mg $^{-1}$] and Ce = the concentration of the herbicide in solution at equilibrium [mg ml^{-1}]. Greater Kd values indicate greater herbicide sorption by the soil relative to the smaller Kd values.

9.4.10 Mathematical and Statistical Analysis

The mineralization activity of the ^{14}C-trifluralin was conducted by measuring the ^{14}C that evolved as carbon dioxide and calculating it as a percent of the radioactivity that was initially added to the soil samples. A first order curve fitting was performed by SigmaPlot software (1986-2000 SPSS Inc.) by use of the following equation.

$$A_t = A_F(1\text{-}e^{-K_F t}) \qquad \textbf{Equation 9.2}$$

Where At = percent degradation at time t (days), AF = percent of added ^{14}C that has evolved at time infinity for the first order curve, and KF = the degradation rate constant (days $^{-1}$) for the first order curve.

The K values that were determined by curve fitting were then used to calculate the half-life for glyphosate in each of the sampled plots. The half-life is calculated based on the percent of the chemical that was mineralized, therefore the half-life presented here is an indication of the time it takes for 50% of the mineralizable fraction to be mineralized. The following equation was used.

$$t^{1/2} = \ln 2/K \qquad \textbf{Equation 9.3}$$

SigmaStat 2.03 software (1995 Access Softek) was used to perform a one-way analysis of variance (ANOVA) on the sorption values, the half-life and the mineralization rates of glyphosate obtained from the experiments. The mean comparisons analyzed in the study and were separated according to the Fisher LSD method at alpha = 0.05.

9.5 Results

9.5.1 Sorption Study

The results of the sorption experiments showed that glyphosate was strongly bound onto the soil constituents regardless of the treatment type (Table 9.1 and 9.2). The lowest sorption value belonged to the soils amended with 10% humic acid (95.9%) and the highest values belonged to the control soils and the soils amended with 2% humic acid (97.4%).

The additions of 10% humic acid to the soil samples significantly affected the percentage of glyphosate sorption in soil ($P = 0.042$) however the Kd values were not significantly different among the treatment groups ($P = 0.200$). The large variability in Kd was because the sorption values were very high. The soil mineral fraction has a greater effect of glyphosate sorption than soil organic matter as noted by the very low R^2 value (0.098) in relation to soil organic matter observed in Chapter 7 of this study and may account for the lowest sorption values in the soils with 10% HA.

Table 9.1 Glyphosate sorption in soil amended with humic acid. Kd [ml g^{-1}] was determined by batch equilibrium experiments.

Treatment	% Sorption	Kd
Control	**97.4a*# (+/- 0.42)**	**114a* (+/- 19.1)**
2% Humic acid	**97.4a (+/- 0.84)**	**119a (+/- 46.5)**
10% Humic acid	**95.9b (+/- 0.15)**	**70a (+/- 2.52)**

*Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at $P < 0.05$ (One-way ANOVA).

The amount and type of fertilizer amendment did not significantly affect glyphosate sorption by soil (P = 0.623). (Table 9.2). The soils amended with P fertilizer exhibited the lowest sorption values (96.5%) while the control soils had the highest values (97.4%). The Kd values were lowest for the 400 kg/ha soils (83) and highest for the control soils (114). The mean differences among the treatment groups were not significantly different (P = 0.397).

Table 9.2 Glyphosate sorption in soil amended with N and P fertilizer. Kd [ml g^{-1}] was determined using batch equilibrium experiments.

Treatment	% Sorption	Kd
Control	**97.4a*# (+/- 0.42)**	**114a*(+/- 19.1)**
30 kg/ha N fertilizer	**97.2a (+/- 0.51)**	**106a (+/- 19.6)**
300 kg/ha N fertilizer	**96.7a (+/- 0.44)**	**90a (+/- 11.1)**
40 kg/ha P fertilizer	**96.5a (+/- 1.44)**	**108a (+/- 30.6)**
400 kg/ha P fertilizer	**96.6a (+/- 0.06)**	**83a (+/- 1.73)**

*Means of three replicates followed by standard deviation.
#Means followed by the same letter are not significantly different at P < 0.05 (One-way ANOVA).

9.5.2 Mineralization Study

The soil test results showed that the amount of added fertilizer had not decreased during the incubation period ensuring the original fertilizer rates were sustained during the incubation period (Table 9.3). There was a slight increase in the amounts for the 40 kg/ha N, the 30 kg/ha P and the control soil and may be the result of variability in the extractions tests. The soil sub samples subjected for N and P analysis were obtained from

the original incubated samples. Variability in soil nutrient content is commonly found in field soils even though the samples are obtained within centimeters of each other (Racz, 1997). One soil sample was used as a control for the P analysis.

Table 9.3 Fertilizer amounts extracted from microcosm soil samples.

Fertilizer Rates	Soil Test Results (ug/g soil)	
	Day 0	Day 14
Control P	49	65
40 kg/ha N (38 ug/g soil)	116	126
400 kg/ha N (380 ug/g soil)	>400	>400
30 kg/ha P (18 ug/g soil)	55	79
300 kg/ha P (180 ug/g soil)	190	190

The addition of humic acid increased the organic carbon in the soil in relation to the control sample (Table 9.4). The amount of organic carbon in the soils amended with 10% HA was more than twice the amount of the control samples. There was very little change in the amounts of soil organic carbon detected in the samples during the two-week incubation period. There were significant differences among the treatment groups at the end of the incubation period ($P = 0.001$).

Table 9.4 Organic carbon detected from microcosm soil samples.

Soil Sample	Day 0 (%Organic-C)	Day 14 (% Organic-C)
Control	**3.34**	**3.31a*# (+/- 0.018)**
2% Humic Acid	**3.67**	**3.62b (+/- 0.005)**
10% Humic Acid	**6.88**	**7.10c (+/- 0.155)**

*Means of two replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

The mineralization of glyphosate proceeded without a lag phase in all of the soil samples amended with humic acid and N and P (Figure 9.1). Initially, the herbicide mineralization proceeded rapidly for all samples but slowed down considerably after three days for the soils amended with 300 kg/ha N and 10% humic acid. Mineralization of the herbicide in the remaining soil samples noticeably declined after three weeks. The increase in the mineralization rate after day 80 can be attributed to the addition of 1 ml of water to the soil samples.

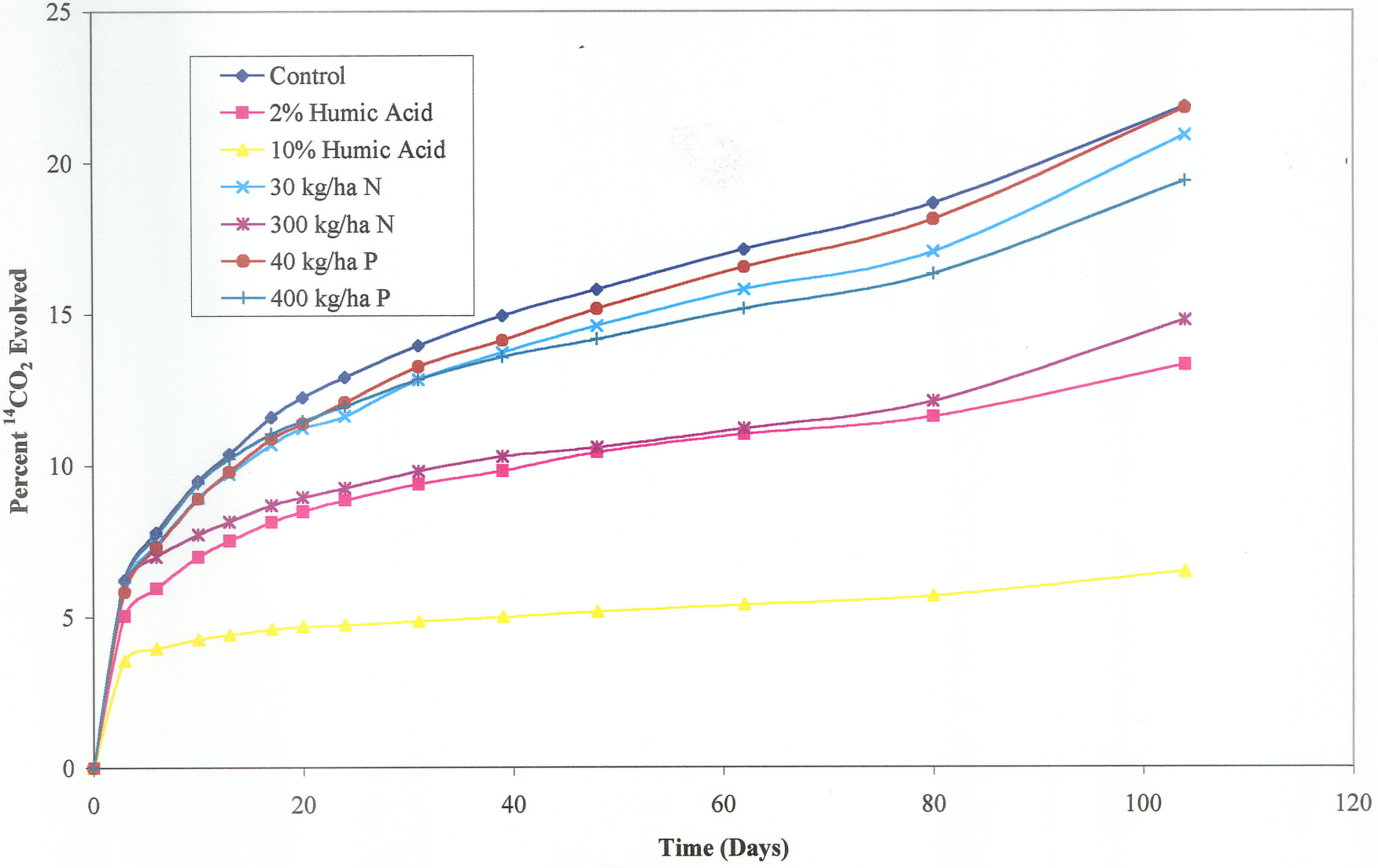

Figure 9.1 Mineralization of glyphosate in soils treated with N and P fertilizers and humic acid. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

The addition of humic acid to the soil samples produced significant differences in the mineralization amounts of glyphosate ($P < 0.001$) (Table 9.5). The highest amounts of mineralization occurred in the control sample (21.8%) and the lowest occurred in the soil treated with 10% of humic acid (6.5%). The half- life of glyphosate in soil ranged from 2.5 days in the 10% HA treatments to 11.6 days in the control treatments and were significantly different ($P = 0.001$) (Table 9.5).

Table 9.5 Mineralization of glyphosate in soil after humic acid additions at 104 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Treatment	Half-life (Days)	$^{14}CO_2$ Evolved (%)
Control	**11.6*#a (+/- 4.114)**	**21.8a*# (+/- 0.320)**
2% Humic acid	**7.3b (+/- 0.200)**	**13.3b (+/- 0.419)**
10% Humic acid	**2.5c (+/- 0.316)**	**6.5c (+/- 0.350)**

*Means of three replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANOVA).

The highest amounts of mineralization occurred in the control sample (21.8%) and the lowest rates occurred in the samples amended with 300 kg/ha of N (14.8%) (Table 9.6). The addition of inorganic N and P fertilizers at the highest rates produced significant differences in the mineralization rates of glyphosate ($P < 0.001$). The half-life of glyphosate in soil ranged from 5.8 days in the 300kg/ha treatments to 13.3 days in the 40 kg/ha treatments and were significantly different ($P = 0.001$) (Table 9.6).

Table 9.6 Mineralization of glyphosate in soil after fertilizer application at 104 days. Calculated as the cumulative percent of added radioactivity recovered as $^{14}CO_2$.

Treatment	Half-life (Days)	$^{14}CO_2$ Evolved (%)
Control	11.6*# a(+/- 4.114)	21.8a*# (+/- 0.320)
30 kg/ha N	11.9a (+/- 1.026)	20.9ac (+/- 0.998)
300kg/ha N	5.8b (+/- 1.389)	14.8bd (+/- 0.802)
40 kg/ha P	13.3a (+/- 0.764)	21.8a (+/- 0.824)
400 kg/ha P	8.9c (+/- 1.026)	19.4ce (+/- 0.901)

*Means of three replicates followed by standard deviation.
#Means followed by the same letter are not statistically different at $P < 0.05$ (One-way ANONA followed by the Tukey test).

9.6 Discussion

The results of this study showed that glyphosate was strongly bound to all soil samples, regardless of treatment. The only significant difference occurred in the soil treated with 10% humic acid, which suggests that the mineral fraction is more important for glyphosate sorption (Sprankle et al., 1975; Glass, 1987). However, the significance of the results seem irrelevant as sorption in the soil treated with 10% HA was only 1.5% less than in the control sample.

The results of this study show that glyphosate underwent microbial degradation in the soil environment as evidenced by the evolution of $^{14}CO_2$ in the soil microcosms. Mineralization of the herbicide occurred without a lag phase in each treatment indicating that a microbial population capable of degrading the herbicide was residing in the soil.

The increase in herbicide mineralization after day 70 can be attributed to the addition of 1 ml of water to the soil samples.

The sharp decrease in glyphosate mineralization after three days in the soils treated with 2% and 10% HA suggests that the soil microorganisms were obtaining their P requirements through sources other than glyphosate. Soil sorption may be ruled out as a factor since glyphosate sorption was significantly the lowest in the soils treated with 10% HA yet these samples exhibited the least amount of herbicide mineralization. Soil bacterial species such as *Pseudomonas* and *Bacillus*, which are capable of metabolizing glyphosate, utilize organic inositol phosphates as their P source, and these compounds are associated with the humic acid fraction in soil (Stevenson, 1994; Paul and Clark, 1996). The HA amendment had a C:N of 64:1 while the C:N ratio of bacteria is commonly 5 to 8:1 (Paul and Clark, 1996). The growth of a soil microbial population may progress slowly under a nitrogen-limiting environment (Miller and Donahue, 1990). However, this does not explain the sudden decrease in glyphosate mineralization in the soils amended with HA. For mineralization to be drastically curtailed, the microbial population would have to experience a sharp decline. This would appear unlikely in the microcosms as the microorganisms inhabited a warm, moist environment, which was conducive for growth.

The significant decrease in glyphosate mineralization in soil amended with 300 kg/ha of fertilizer N is likely due to the toxic effects of the urea fertilizer (Michel and Mew, 1998). During urea hydrolysis, a soil solution with pH > 9 is formed at the reaction site and can decrease microbial populations in soil. The reduction in glyphosate metabolism was not significantly different at 30 kg/ha from the control sample, suggesting that urea toxicity only occurs at high application rates. Soil sorption of glyphosate at the high rate

of N was not significantly different from the control soil and therefore can be ruled out as affecting mineralization of the herbicide.

The mineralization of glyphosate was significantly lower in the samples treated with 400 kg/ha of P fertilizer suggesting that the soil microorganisms may be obtaining their P requirements from sources other than glyphosate. Studies have shown that soil microorganisms cultured in soils containing phosphate preferentially degrade the compound over glyphosate and this may account for this observation (Fitzgibbon and Braymer, 1988; Dick and Quinn, 1995). The soils treated with 40 kg/ha of fertilizer P exhibited identical mineralization rates as the control soil suggesting typical rates of P will not affect glyphosate mineralization in soil. Soil sorption of glyphosate was very high and not significant in the P amended soils, therefore it should be ruled out as affecting the mineralization of the herbicide.

The half-life of glyphosate in the soils treated with HA and N and P fertilizers were significantly different. However, they were the lowest in the treatments (10% HA and 300 kg/ha N), which also exhibited the lowest mineralization rates, and are indicative that initially a portion of the glyphosate is readily mineralized.

9.7 Summary and Conclusions

The sorption study showed that glyphosate was strongly bound to all soils irrespective of humic acid or fertilizer treatment. However, sorption behavior alone cannot account for the varied mineralization rates among the various treatment groups.

The soils amended with HA and high rates of N and P fertilizers significantly affected the mineralization of glyphosate in soil. The soil microorganisms may have preferentially

degraded the organic P compounds associated with the HA in the soil. The soils amended with high rated of urea N fertilizer may have been toxic to the soil microorganisms thereby limiting their metabolic activity. Whereas in the soils amended with high rates of P fertilizer, the soil microbes may have preferentially degraded the phosphate fertilizer over glyphosate. The half-life of glyphosate in soil was the lowest in the soil amended with 10% humic acid and highest in the soil amended with 40 kg/ha of P fertilizer.

CHAPTER 10

General Discussion

The purpose of this study was to determine whether short-term changes in crop rotations, or fertilizer and humic acid additions to the soil would have an impact on the fate of trifluralin and glyphosate in the soil environment. These herbicides were chosen as they are commonly applied in Manitoba field crops. The project consisted of both field and laboratory measurements dealing with extraction of trifluralin from field soils, and laboratory experiments on the sorption and desorption behavior and mineralization of these herbicides in soil.

10.1 Study Results

10.1 Comparisons of Trifluralin and Glyphosate

The sorption/desorption values for trifluralin and glyphosate were remarkably similar. Both herbicides exhibited very strong sorption onto the soil constituents, with negligible desorption occurring. Although the treatments for glyphosate also included nitrogen and phosphate fertilizers and humic acid, the values were not significantly different from the soils collected from the rotational plots. These results were in agreement with results from previous studies (Helling, 1976; Nomura and Hilton, 1976), however the glyphosate samples treated with inorganic phosphates deviated from previous research as the

fertilizer amendments did not significantly affect glyphosate sorption (de Jonge and de Jonge, 1999). This study utilized realistic phosphate application rates and therefore may better reflect what may happen in agricultural field conditions.

The mineralization rates were much lower over the 168 day incubation period for trifluralin than for glyphosate. These results were in accordance with other studies (Wheeler et al., 1979; and Smith and Aubin, 1992) that utilized soil microcosms to evaluate the mineralization of these herbicides in the soil environment. Trifluralin is noted for its persistence in soil as a number of studies have shown that the herbicide may persist for two years or more after application (Corbin et al., 1994; Gerwin and McKercher, 1992). The soils from the MCDC plots showed extractable amounts of trifluralin eleven months after field application further illustrating field persistence of the herbicide. However, the amounts detected in the MCDC samples would not likely injure crops grown the year following the sampling date.

The mineralization of glyphosate from the non-amended field soil was much higher than soils receiving high amounts of N fertilizer and HA additions. Although the amendments were unrealistic compared to actual field applications, the data suggest that farm management practices may impact the behavior of glyphosate in the soil.

Herbicide sorption is often linked to herbicide degradation rates, as higher sorption decreases the mineralization (Scow, 1993). In this study, the sorption values for both herbicides approached 100%, however, the mineralization of glyphosate was approximately five times that of trifluralin. Therefore, sorption alone cannot account for the mineralization behavior of the herbicides.

The soil organic matter contents were very similar for the experiments conducted on trifluralin and glyphosate. The R^2 values for glyphosate with respect to SOM were negligible, but they were moderate for trifluralin suggesting greater binding for trifluralin on the SOM, which this may account for the lower mineralization amount of trifluralin compared with glyphosate. Therefore, the binding of glyphosate would rely more on the soil mineral content. Consequently, glyphosate may have been more accessible to the soil microbes, whereas the organic molecules may have provided a temporary hiding place for the trifluralin molecules, hence less decomposition.

10.2 Field versus Laboratory Studies

The bulk of the experimental work for this study was conducted in the laboratory. A number of differences can be noted of how laboratory studies may not mimic actual field conditions. For the sorption experiments, the soil samples were air-dried whereas in the field a range of moisture conditions may exist. The soil microbial population would be less active in dry soils whereas in the field, the herbicide mineralization by an active group of microorganisms, under ideal moisture conditions, may readily degrade a portion of the chemical and therefore different sorption values may be produced. The soil samples in the centrifuge tubes were saturated with the herbicide solution (5 g soil, 15 ml solution), thereby achieving a sorption environment that would not likely occur in a field environment, except during heavy rainfall events, in which case the herbicide may either be leached through the soil profile or be transported by surface runoff events. Throughout the sorption procedure a uniform temperature was maintained (20°C) while under field conditions the soil would be subjected to a range of environmental temperatures, which

may also impact the sorptive ability of the herbicide as well as the activity of the soil microorganisms.

The soil microcosms utilized in this study are not indicative of actual field conditions. The soil microorganisms were subjected to an environment that contained adequate water (field capacity) and a constant temperature of 20°C. Actual field conditions are subjected to a range of moisture and temperature regimes that may impact microbial activity. Cold soils and desiccated soils, which may occur in the field, would restrict the activity of the microorganisms and therefore their ability to degrade organic compounds such as pesticides.

The soil microcosms in this study quantified the mineralization of glyphosate by the evolution of $^{14}CO_2$. However, the liberation of $^{14}CO_2$ does not necessarily reflect the actual rate of decomposition of ^{14}C-glyphosate (Smith and Aubin, 1993). In the radioactive herbicide used in this study the ^{14}C label was in the phosphonomethyl carbon atom. This carbon atom is also present in aminomethylphosphonic acid, the major soil metabolite of glyphosate. Thus, the $^{14}CO_2$ evolved could have been released by the direct metabolism of ^{14}C-glyphosate and/or by metabolism of ^{14}C-containing degradation products.

There are two other factors that may confound the mineralization results from the soil microcosm (Brady, 1996). Some of the $^{14}CO_2$ produced by the soil microbes reacts with the soil solution and produces H_2O_3, but in very small quantities. In a field situation, there is a constant flux of gaseous O_2 into the soil resulting in an aerobic soil environment. The microcosm jars were sealed for most of the duration of the experiment, eliminating the constant flux of O_2 from the atmosphere. This may have increased the formation of

anaerobic micro sites in the soil. Under highly anaerobic conditions, CH_4 and CS_2 may be produced (Brady, 1996). The NaOH traps cannot detect these metabolic products.

Pseudomonads, Rhizobia, and *Arthrobacter* which are capable of degrading glyphosate and trifluralin, are classified as rhizobacteria, which form close associations with the rhizosphere, or the area immediately surrounding the plant roots (Paul and Clarke, 1996). Microbial counts increase significantly in this soil region because of the wide range of organic materials provided by the roots that act as substrates for the microorganisms. Consequently, studies have shown that herbicide degradation is more efficient in the rhizosphere than in the bulk soils (Piutti et al., 2002). The soil microcosms in this study did not contain growing plants. As a result, the number of herbicide degraders colonizing the soil environment may be much less than if the soil were in a natural cropping environment. As such, the extrapolation of data contained in this study to field soils should be done with caution.

10.3 Possible Error in Total Recovery of Radioactivity

There are two errors for that may have occurred while quantifying $^{14}CO_2$ evolution during this study:

(1) A 10 ml solution of 1 M NaOH was used to trap the carbon dioxide that evolved from the soils. A commercially available scintillation cocktail was used during the process of liquid scintillation counting to determine the level radioactivity associated with each trap. Voroney et al. (1991) claim that premixed scintillation cocktails have an 85% efficiency rate for counting $^{14}CO_2$ absorbed in NaOH. Error may have occurred due to this efficiency level.

(2) During the process of trap changing, a small amount of $^{14}CO_2$ may have been lost when the microcosms were temporally opened. Although small losses may be associated with each change, numerous trap changes occurred during the course of the experiment.

The monitoring of $^{14}CO_2$ radioactivity is a common practice for quantifying the microbial degradation of organic compounds, As such, despite the two reasons presented as possible shortcomings of the method, research utilizing this procedure is relevant for assessing the degradation of organic compounds in the environment.

CHAPTER 11

Summary and Conclusions

Generally, the types of crop rotation and crop grown did not significantly impact the behavior of trifluralin and glyphosate in the soil environment. Both herbicides were strongly sorbed by soil irrespective of the type of crop grown or the type of crop rotation. The sorption of glyphosate was not significantly affected in the soils amended with nitrogen and phosphate fertilizers, however, significant differences were found in the soils amended with high rates of humic acid. Trifluralin sorption in soil was moderately correlated to the amount of organic carbon in soil while glyphosate was not. Therefore, crop rotations and management practices may impact the sorption behavior of trifluralin and glyphosate in soil. Crops that contribute higher SOM such as cereals, and reduced tillage practices, will increase SOM inputs, therefore increasing trifluralin sorption in relation to glyphosate in soil.

The mineralization rates of glyphosate were approximately four times greater than for trifluralin. The soils amended with high amounts of N fertilizer significantly decreased the mineralization of glyphosate in the soil, apparently due to the toxicity affect on the soil microorganisms (Michel and Mew, 1998). A significant decrease in the mineralization of glyphosate was also found in the soils amended with high amount of humic acid. This was attributed to the soil microorganism preferentially degrading the labile organic phosphate associated with the humic acid molecule (Paul and Clark, 1996).

Mineralization of glyphosate was also significantly decreased in the soils amended with high rates of P fertilizer and may be due to the microorganisms preferentially degrading the phosphate molecule (Dick and Quinn, 1995; Fitzgibbon and Braymer, 1998).

The results of this study support previous studies that trifluralin and glyphosate are strongly sorbed in soil (Piccolo et al., 1996; de Jonge et al., 2001; Malterre et al., 1997; Pederson et al., 1995). This behavior will therefore mitigate the off-site movement of the herbicides, (except movement by soil erosion) resulting in the mitigation of environmental contamination to surface and ground waters, the atmosphere, and therefore, non-target organisms.

The results of this study are supported by previous studies that show that trifluralin and glyphosate undergo microbial degradation in soil (Nomura and Hilton, 1977; Smith and Aubin, 1992; Parr and Smith, 1971; Solbakken et al., 1982). And the studies by Wheeler et al., (1979) and Nomura and Hilton, (1976) also lend support to this study, showing that mineralization of glyphosate generally proceeds at a faster rate than trifluralin.

The potato rotation study at MCDC had completed its third year and therefore the type of rotation and crops grown in the rotation was not long enough to significantly impact the chemical and physical properties of the soil. This study also showed that nitrogen levels and organic matter content in soil could influence trifluralin and glyphosate fate processes in soil. Therefore, further study is needed as the rotation progresses in order to assess the behavior of the herbicides trifluralin and glyphosate in the soil.

CHAPTER 12

Contribution to Knowledge

This study has found that high rates of P fertilizer did not impact the sorption of glyphosate in the soil. Therefore, continued application of P fertilizers on agricultural soils would not decrease the persistence of this herbicide in the soil. However, high rates of P fertilizer may slow down the degradation of glyphosate in soil as the microbial species responsible may utilize the fertilizer P more readily than the P associated with the glyphosate molecule.

This study has shown that sorption alone cannot account for the different mineralization rates of glyphosate in soil. Other factors such as N toxicity and P availability associated with HA may slow down the mineralization of the herbicide.

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APPENDICES

I. Results of the analysis of variance.

Table 1. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation and the soil extraction of trifluralin from the MCDC plots.

Source	DF	SS	MS	F Ratio	P Value
Model	3	0.074	0.025	1.59	0.265*
Error	8	0.124	0.015		
Total	11	0.198	0.018		

*Significant at $P < 0.05$.

Table 2. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on soil organic matter content in soil (Trifluralin sorption).

Source	DF	SS	MS	F Ratio	P Value
Model	5	1.502	0.300	2.964	0.044*
Error	16	1.621	0.101		
Total	21	3.123	0.149		

*Significant at $P < 0.05$.

Table 3. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on soil sorption of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	5	0.335	0.067	1.755	0.179*
Error	16	0.610	0.038		
Total	21	0.945	0.045		

*Significant at $P < 0.05$.

Table 4. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on Kd of soil sorption of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	31583	6319	1.638	0.207*
Error	16	61716	3857		
Total	21	93309	4443		

*Significant at $P < 0.05$.

Table 5. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on soil desorption of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.196	0.039	2.100	0.119*
Error	16	0.298	0.019		
Total	21	0.494	0.024		

*Significant at $P < 0.05$.

Table 6. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on soil organic matter content in soil (Trifluralin sorption).

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.975	0.487	4.310	0.029*
Error	19	2.148	0.113		
Total	21	3.123	0.149		

*Significant at $P < 0.05$.

Table 7. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on soil sorption of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.285	0.142	4.094	0.033*
Error	19	0.660	0.035		
Total	21	0.945	0.045		

*Significant at $P < 0.05$.

Table 8. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on Kd of soil sorption of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	24985	12493	3.474	0.052*
Error	19	68323	3596		
Total	21	93309	4443		

*Significant at $P < 0.05$.

Table 9. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on soil desorption of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.0005	0.0002	0.008	0.992*
Error	19	0.5437	0.0259		
Total	21	0.5442	0.0259		

*Significant at $P < 0.05$.

Table 10. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on trifluralin mineralization in soil after 20 days.

Source	DF	SS	MS	F Ratio	P Value
Model	5	0.137	0.027	1.873	0.156*
Error	16	0.233	0.015		
Total	21	0.370	0.017		

*Significant at $P < 0.05$.

Table 11. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on trifluralin mineralization in soil after 105 days.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.398	0.080	2.246	0.100*
Error	16	0.567	0.035		
Total	21	0.964	0.046		

*Significant at $P < 0.05$.

Table 12. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on trifluralin mineralization in soil after 168 days.

Source	DF	SS	MS	F Ratio	P Value
Model	5	0.528	0.106	3.128	0.370*
Error	16	0.541	0.034		
Total	21	1.069	0.051		

*Significant at $P < 0.05$.

Table 13. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on trifluralin mineralization in soil after 20 days.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.085	0.0002	1.516	0.245*
Error	19	0.532	0.0259		
Total	21	0.617	0.0259		

*Significant at $P < 0.05$.

Table 14. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on trifluralin mineralization in soil after 105 days.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.215	0.107	2.736	0.090*
Error	19	0.746	0.040		
Total	21	0.961	0.046		

*Significant at $P < 0.05$.

Table 15. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on trifluralin mineralization in soil after 168 days.

Source	DF	SS	MS	F Ratio	P Value
Model	2	0.342	0.171	4.467	0.026*
Error	19	0.727	0.038		
Total	21	1.069	0.051		

*Significant at $P < 0.05$.

Table 16. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on the half-life of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	5	565	113.1	0.796	0.568*
Error	16	2273	142.0		
Total	21	2838	135.1		

*Significant at $P < 0.05$.

Table 17. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on the half-life of trifluralin in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	247.9	123.9	0.909	0.420*
Error	19	2590	136.3		
Total	21	2838	135.1		

*Significant at $P < 0.05$.

Table 18. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on the volatilization of trifluralin from soil.

Source	DF	SS	MS	F Ratio	P Value
Model	5	6.033	1.207	1.244	0.335*
Error	16	15.52	0.970		
Total	21	21.56	1.027		

*Significant at $P < 0.05$.

Table 19. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on the volatilization of trifluralin from soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	2.892	1.446	1.472	0.255*
Error	19	18.66	0.982		
Total	21	21.56	1.027		

*Significant at $P < 0.05$.

Table 20. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on the soil organic content (Glyphosate sorption).

Source	DF	SS	MS	F Ratio	P Value
Model	13	1.163	0.090	0.4805	0.858*
Error	4	0.745	0.186		
Total	17	1.908	0.112		

*Significant at $P < 0.05$.

Table 21. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on the sorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	13	0.990	0.076	1.455	0.212*
Error	22	1.523	0.053		
Total	35	2.143	0.061		

*Significant at $P < 0.05$.

Table 22. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on the Kd of soil sorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	13	11118168	855244	2.148	0.055*
Error	22	8759190	398145		
Total	35	19877358	567925		

*Significant at $P < 0.05$.

Table 23. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on desorption of glyphosate from soil.

Source	DF	SS	MS	F Ratio	P Value
Model	13	2.510	0.193	6.436	<.0001*
Error	22	0.660	0.030		
Total	35	3.170	0.091		

*Significant at $P < 0.05$.

Table 25. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on soil organic content.

Source	DF	SS	MS	F Ratio	P Value
Model	4	0.806	0.202	2.380	0.105*
Error	13	1.102	0.085		
Total	17	1.908	0.112		

*Significant at $P < 0.05$.

Table 26. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on the sorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	4	0.245	0.062	1.270	0.332*
Error	13	0.630	0.048		
Total	17	0.880	0.052		

*Significant at $P < 0.05$.

Table 27. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on the Kd of sorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	4	480094	120024	1.527	0.252*
Error	13	1022160	78628		
Total	17	15022454	88368		

*Significant at $P < 0.05$.

Table 28. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on the desorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	4	0.515	0.129	1.200	0.157*
Error	13	0.845	0.065		
Total	17	1.360	0.080		

*Significant at $P < 0.05$.

Table 29. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of phosphate applications to glyphosate sorption in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	5	0.824	0.165	5.192	0.009*
Error	12	0.381	0.032		
Total	17	1.205	0.071		

*Significant at $P < 0.05$.

Table 30. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of phosphate applications on Kd of soil sorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	5	213986429	42797286	3.774	0.028*
Error	12	136066518	11338876		
Total	17	350052947	20591350		

*Significant at $P < 0.05$.

Table 31. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on glyphosate mineralization from soil after 21 days.

Source	DF	SS	MS	F Ratio	P Value
Model	13	16.71	1.285	1.285	0.006*
Error	40	18.47	0.462		
Total	53	35.18	0.664		

*Significant at $P < 0.05$.

Table 32. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on glyphosate mineralization from soil after 105 days.

Source	DF	SS	MS	F Ratio	P Value
Model	13	32.56	2.505	5.904	<.0001*
Error	40	16.97	0.424		
Total	53	49.53	0.934		

*Significant at $P < 0.05$.

Table 33. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on glyphosate mineralization from soil after 168 days.

Source	DF	SS	MS	F Ratio	P Value
Model	13	39.70	3.053	7.095	<.0001*
Error	40	17.21	0.430		
Total	53	57.91	1.074		

*Significant at $P < 0.05$.

Table 34. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on glyphosate mineralization from soil after 21 days.

Source	DF	SS	MS	F Ratio	P Value
Model	5	7.670	1.917	3.420	0.015*
Error	49	27.51	0.561		
Total	53	35.18	0.664		

*Significant at $P < 0.05$.

Table 35. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on glyphosate mineralization from soil after 105 days.

Source	DF	SS	MS	F Ratio	P Value
Model	5	10.22	2.555	3.186	0.021*
Error	49	39.31	0.802		
Total	53	49.53	0.934		

*Significant at $P < 0.05$.

Table 36. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on glyphosate mineralization from soil after 168 days.

Source	DF	SS	MS	F Ratio	P Value
Model	5	15.55	3.890	4.500	0.004*
Error	49	42.35	0.864		
Total	53	57.90	1.092		

*Significant at $P < 0.05$.

Table 37. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop rotation on glyphosate half-life in soil after 168 days.

Source	DF	SS	MS	F Ratio	P Value
Model	13	47.45	3.650	1.119	0.505*
Error	4	13.05	3.263		
Total	17	60.09	3.559		

*Significant at $P < 0.05$.

Table 38. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of crop grown on glyphosate half-life in soil after 168 days.

Source	DF	SS	MS	F Ratio	P Value
Model	4	18.52	4.631	1.434	0.278*
Error	13	41.97	3.229		
Total	17	60.50	3.559		

*Significant at $P < 0.05$.

Table 39. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of HA additions to mineralization of glyphosate in soil after 104 days.

Source	DF	SS	MS	F Ratio	P Value
Model	2	355.8	177.9	1334	0.0001*
Error	6	0.800	0.133		
Total	8	356.6	44.57		

*Significant at $P < 0.05$.

Table 40. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of fertilizer additions to glyphosate mineralization in soil after 104 days.

Source	DF	SS	MS	F Ratio	P Value
Model	4	103.8	25.96	40.16	0.0001*
Error	10	6.463	0.646		
Total	14	110.3	7.878		

*Significant at $P < 0.05$.

Table 41. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of HA additions to glyphosate sorption in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	4.187	2.093	6.408	0.042*
Error	5	1.633	0.327		
Total	7	5.820	0.831		

*Significant at $P < 0.05$.

Table 42. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of HA additions to Kd on sorption of glyphosate in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	2	4241	2120	2.255	0.200*
Error	5	4702	940.4		
Total	7	8944	1278		

*Significant at $P < 0.05$.

Table 43. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of N and P fertilizer additions to glyphosate sorption in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	4	1.589	0.397	0.679	0.623*
Error	9	5.260	0.584		
Total	13	6.859	0.527		

*Significant at $P < 0.05$.

Table 44. DF, SS, F ratio and P levels of the one-way analysis of variance on the effect of N and P fertilizer additions to Kd of glyphosate sorption in soil.

Source	DF	SS	MS	F Ratio	P Value
Model	4	1651	412.7	1.141	0.397*
Error	9	3257	361.8		
Total	13	4907	377.5		

*Significant at $P < 0.05$.