THE PHYTOTOXIC EFFECT OF ALS INHIBITING HERBICIDE COMBINATIONS IN PRAIRIE SOILS

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Abstract

The objective of this study was to determine if the presence of two ALS inhibiting herbicide residues in three Saskatchewan soils would result in an additive, synergistic, or antagonistic interaction. This was determined through field trials where herbicides were applied sequentially over the course of two years and through dose-response modelling. The herbicides examined in these experiments were imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam, each in combination with imazamox/imazethapyr. The phytotoxicity and persistence of the herbicides in soil was assessed using an Oriental mustard root inhibition bioassay. The determination of herbicide interaction was made through the comparison of the experimentally observed values to theoretically expected values derived from a mathematical equation.

The dose response curves created by placing incremental concentrations of these herbicides in soil were compared using the I_{50} parameter, which is the concentration resulting in a 50% reduction in root length. It appeared that soil organic matter followed by soil pH had the greatest effect in reducing herbicide residue phytotoxicity in the tested soils. Based on the bioassay analysis of sequentially applied ALS inhibiting herbicides, it is proposed that the phytotoxic effect of herbicide residues in soil result in additive injury effects rather than synergistic or antagonistic interactions.

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1. INTRODUCTION

Acetolactate synthase (ALS) inhibiting herbicides are an important group of herbicides, some of which have some soil residual activity after the time of application. This group of herbicides include a number of compounds that can be applied to broadleaf or grass crops for control of a broad spectrum of weed species. Good crop tolerance and weed control, along with low application rates and low mammalian toxicity have contributed to an increase in popularity of these herbicides (Brown 1990; Vencill 2002).

ALS inhibitors are highly plant active through both foliage and root uptake. This ability to be active in the soil and be taken up through the root system is beneficial for the control of weeds that emerge after the date of application. In years of reduced herbicide degradation in the soil due to reduced temperatures or soil moisture, some ALS inhibitors or their metabolites can persist into the following growing seasons (Hall et al. 1999; Hill et al. 1998). This prolonged persistence can potentially injure sensitive crops grown in rotation such as canola and lentils (non-Clearfield® varieties), mustard, or sugar beet (Onofri 1996; Moyer and Esau 1996; Moyer and Hamman 2001).
Numerous factors influence persistence of these herbicides. Clay content, organic matter content, soil pH, landscape position, microbial populations, and tillage regimes all can influence the sorption and degradation of these herbicides in the soil along with moisture levels and temperatures (Ayeni et al. 1998; Krieger et al. 2000; Moyer and Hamman 2001; Schoenau et al. 2005).

The wide range of crops that can be treated with this group of herbicides can result in some repeated applications on the same land. The concern is that if these herbicides persist into the following growing season and another herbicide from the same group is applied, which also has soil active properties, do the two compounds interact with each other in the soil? This is possible because the application of some soil insecticides was found to reduce crop tolerance to post-emergent herbicides in corn (Kapusta and Krause 1992; Diehl et al. 1995). Another consideration of this possible

interaction is whether or not this could ultimately affect the growth of the next sensitive species grown in rotation.

The objectives of the research were:

1) Develop dose response models based on a root inhibition bioassay and determine the behaviour and interactions of the herbicides applied together in the lab.
2) Determine if the sequential applications of ALS inhibiting herbicides in the field over two years would interact to form synergistic, antagonistic, or additive responses.

2. LITERATURE REVIEW

2.1 ALS Inhibiting Herbicides: Classification and Mode of Action

ALS inhibiting herbicides are those that inhibit plant growth by inhibiting acetolactate synthase (ALS), also known as acetohydroxy acid synthase (AHAS), an enzyme (EC 4.1.3.18) required for the biosynthesis of the branched-chain amino acids leucine, isoleucine, and valine (Brown 1990, Hall et al. 1999). These herbicides are relatively unique in their ability to control weeds from direct application to the plant or through application to the soil where they are still biologically active. These herbicides can therefore be absorbed through the foliage or roots and have high mobility in plants. This provides control of emerged weeds in crop, and also provides control of weeds that emerge after the time of application (Vencill 2002).

The ALS inhibitors include compounds in the imidazolinone, sulfonylurea, triazolopyrimidine sulfonanalide, pytimidinylthiobenzoate, and sulfonylaminocarbonyl-triazolinone chemical families and have many benefits including very low application rates and low mammalian toxicity (Brown 1990; Vencill 2002). These herbicide products were quickly adopted by agricultural producers for crop production because of their broad-spectrum and soil persistence that provides some control of emerging weeds after application. Plant tolerance to ALS inhibitors is due to rapid metabolic inactivation of the chemical (Brown 1990). Death of susceptible species is slow, with growth stopping immediately. Plant death is caused by a combination of amino acid inhibition and the disruption of cell division. Symptoms are reddening of midrib and veins, wilted leaves, chlorosis, and necrosis that first appears in the meristematic regions (Vencill 2002).

2.2 Soil Residual Herbicides

Soil residual herbicides are those compounds that control plant growth through out the growing season due to the persistence of phytotoxic residues in the soil (Helling 2005). Koskinen et al. (2006) stated that sorbed herbicides are not immediately

Table 2.1: Classification of the ALS inhibiting herbicides that were utilized for the experiment in this thesis.

Common Name	Chemical Name	Chemical Family	Trade Name
Florasulam	N-(2,6-difluorophenyl)-8-fluoro-5-methoxy(1,2,4) triazolo(1,5c)pyrimidine -2-sulfonamide	Triazolopyrimidine sulfonanalide	Frontline™
Flucarbazone-sodium	4,5-dihydro-3-methoxy-4-methyl-5-oxo-N-(2-(trifluoromethoxyphenylsulfonyl)) -1H-1,2,4-triazole-1-carboxamide sodium salt	Sulfonylamino carbonyltriazolinone	Everest™
Imazamethabenz	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-4(and 5)-methylbenzoic acid (3:2)	Imidazolinone	Assert™
Imazamox	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid, ammonium salt	Imidazolinone	Odyssey™ (component) Solo™
Imazethapyr	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid	Imidazolinone	Odyssey™ (component) Pursuit™
Sulfosulfuron	1-(4,6-dimethoxypyrimidin-2-yl)-3-(2-ethanesulfonyl-imidazo[1,2-a]pyridine-3-yl)sulfonylurea	Sulfonylurea	Sundance™

Information from Vencill, 2002

available for uptake or degradation, and that the compound must first desorb from the soil. Therefore the amount of herbicide that sorbs to the soil, and the rate it can desorb back into the soil solution determines the overall phytotoxicity of that herbicide.

Herbicides in soil are generally weakly adsorbed to soil aggregates, which allows for persistence because not all the chemical is available for degradation at once. This allows for the accumulation of the herbicide at the soil solution – soil colloid interface (Helling 2005). Additional accumulation of the herbicide or its metabolites takes place with covalent bonding to soil organic matter particles, which also increases soil persistence (Helling 2005). These herbicides are then able to move back into the soil solution becoming available and thus phytotoxic to susceptible species (Hall et al. 1999, Vencill 2002). Damage often appears as root stunting and pruning due to the meristematic region being affected (Vencill 2002).

2.2.1 Imazamox

Imazamox is a herbicide that is not highly residual, with a field half-life determined to be 20 to 30 days and a nonreversible sorption to soil colloids (Vencill 2002). Even though persistence is less likely to be an issue for soil residual imazamox than many other ALS inhibitors, there have been cases where it has persisted with phytotoxic effects on following crops (Cobucci et al. 1998; O'Sullivan et al. 1998).

2.2.2 Imazethapyr

Imazethapyr is weakly and reversibly sorbed to soil, and is quite persistent with a field half-life of 60 to 90 days (Vencill 2002). Imazethapyr is relatively non-mobile in the soil profile, and is reported to remain predominately in the top 15 cm, but will move down as far as 30 cm (Jourdan et al. 1998a; Vencill 2002). Imazethapyr is very phytotoxic even at low doses; phytotoxicity has been reported in crops at residue levels between 0.5 and 3 $\mu g\ kg^{-1}$ of soil (Jourdan et al. 1998b; Bresnahan et al. 2000). Imazethapyr persistence is greatly influenced by soil properties including clay content, organic matter, and pH. With higher clay and organic matter contents, adsorption of imazethapyr is increased. Adsorption removes the herbicide from the soil solution therefore decreasing its phytotoxicity but also making it unavailable for degradation

(Loux and Reese 1993). As the pH of the soil is lowered, this results in the change of imazethapyr from an anionic state to a more neutral state. This allows for more of the herbicide to adsorb to the soil colloid surfaces, resulting in less herbicide in soil solution and available for degradation (Helling 2005; Loux and Reese 1993; Renner et al. 1988). Soil pH also influences the ability of soil bound imazethapyr to desorb back into the soil solution. Bresnahan et al. (2000) found that even though less imazethapyr was adsorbed to soil colloids at a high pH, although the sorbed compound is much more resistant to desorption than at lower pH levels.

2.2.3 Imazamethabenz

Imazamethabenz has a field half-life of 25 to 35 days, meaning that there is the potential for prolonged activity in the soil. This compound will remain in the upper soil profile because there is limited movement in the soil due to its low solubility in water. Therefore all of the phytotoxic residues will remain in the root zone until it is completely degraded. Soil residues of this compound can be highly phytotoxic due to its reversible adsorption to soil colloids, which allows it to become plant available over time (Vencill 2002).

2.2.4 Flucarbazone-sodium

Eliason et al. (2004) reported the field half-life of flucarbazone-sodium to vary from 6 to 110 days in a range of prairie soils. Conventional sunflowers were injured by a quarter of the recommended rate of flucarbazone applied to soil prior to seeding, indicating that residues could be a problem in crop rotations (Howatt and Endres 2006). Although flucarbazone did not reduce the height of the sunflower at this rate, it did reduce plant weight. Flucarbazone adsorption, which influences soil persistence, will increase with higher clay contents and organic matter levels in the soil (Koskinen et al. 2006). Sorption to soil organic matter was reported to reduce phytotoxicity of flucarbazone (Eliason et al. 2004).

2.2.5 Sulfosulfuron

Sulfosulfuron, with a field half-life of 14 to 75 days, is often one of the more persistent herbicides. Though some of the residue is microbially degraded, for the most part hydrolysis is responsible for its chemical breakdown. The persistence of this compound is heavily influenced by rainfall and soil moisture. Residues resulting in phytotoxic effects have been reported from one to three years after application (Vencill 2002).

Some rotational crops are sensitive to sulfosulfuron residues in the soil resulting in reduced biomass or lower yields. Sunflower is one of the sensitive crops, with symptoms including shoot stunting, discoloration and root pruning (Alonso-Prados et al. 2002). Moyer and Hamman (2001) found a positive correlation between the rate of sulfosulfuron that caused a 50% reduction in dry weight and soil organic matter, and a negative correlation between the herbicide rate that caused 50% reduction in dry weight and soil pH levels. Lower levels of soil organic matter and higher soil pH contribute to reduced sorption of sulfonylurea herbicides in the soil. This results in greater phytotoxic responses to susceptible plant species resulting in stunting (Morishita et al. 1985).

2.2.6 Florasulam

Florasulam is weakly adsorbed to soil colloids, and has a relatively short field half-life of 2 to 18 days (Vencill 2002). Jackson et al. (2000) found that the primary metabolite of florasulam, 5-hydroxyflorasulam, has very little plant activity. Increasing temperatures had a significant effect on reducing herbicide persistence (Krieger et al. 2000).

2.3 Phytotoxicity and Rotational Crops

The ability of a soil residual herbicide to have a phytotoxic effect on a sensitive crop in following years depends, in part, on the half-life of the herbicide being used. The half-life of herbicides in soil varies with the chemical structure and soil conditions that affect degradation. Moyer and Esau (1996) found that canola was injured the year after imazethapyr application, sugar beet was injured after imazamethabenz and imazethapyr application, and potatoes not only suffered some yield loss but considerable

quality loss as well. They also found that sugar beets were damaged three years after application of a high rate of imazethapyr. Alonso-Prados et al. (2002) found that sulfosulfuron residues resulted in symptoms including dark green colouration, stunting with a reddening of the stem base, and a less dense secondary root system. Shinn et al. (1998) reported injury to peas, canola, and barley the year after sulfosulfuron application. Soil residual herbicides were reported to have a phytotoxic effect the year following application, with reduced yields of oats, barley, pea, alfalfa, sugar beet, chili, tomato, and cantaloupe resulting from a combination of imazapyr with either imazapic or imazethapyr (Alister and Kogan 2005).

When sulfonylureas are applied, measuring soil organic matter can help to assess the risk of damage to sensitive rotational crops such as canola, pulses, and sugar beets. Soils with < 4% organic matter will likely result in injury to these crops from sulfonyl-urea residues when they are planted one year later. Recropping recommendations can be less restrictive on soils previously treated with a sulfonylurea herbicide and having > 4% organic matter (Moyer and Hamman 2001).

2.4 Factors Influencing Residue Persistence

One of the properties of some ALS inhibiting herbicides is soil residual activity that can result in weed control throughout the growing season. However, this characteristic can also cause crop damage and an economic loss due to a phytotoxic effect on sensitive rotational crops (Cobucci et al. 1998; O'Sullivan et al. 1998). The degree to which a residual herbicide can persist and cause damage is influenced by the soil properties, environmental conditions and landscape position (Ayeni et al. 1998; Krieger et al. 2000; Moyer and Hamman 2001; Schoenau et al. 2005).

2.4.1 Soil Properties

Clay and organic matter (OM) content along with soil pH have a large impact on the fate and toxicity of herbicide residues in soil. The importance of soil properties in persistence and phytotoxicity is dependent upon the residual herbicide being applied (Loux and Reese 1993; Shinn et al. 1998). For example, Loux and Reese (1993) reported that imidazolinone persistence increases with clay and organic matter content.

Soil clay content influences many types of soil active herbicides as higher clay contents were found to increase trifluralin persistence (Gaynor 1985). Koskinen et al. (2002) found that sulfonylamino-carbonyl triazolinone herbicides underwent greater hydrolysis breakdown in sandy loam soils compared to clay loam soils. The increase in hydrolysis was due to reduced adsorption in the sandier soil, resulting in more of the chemical being present in the soil solution.

Variations in soil pH can influence how long a herbicide will persist. The pH of the environment the herbicide is found in influences whether or not the herbicide is in a neutral, anionic, or cationic state. Shaner and Hornford (2005) stated that at a pH greater than six, imidazolinone herbicides tend to be found in the anionic form. The result tends to be more of the herbicide in the soil solution, making it more plant available and therefore phytotoxic but also increasing its degradation and reducing persistence. Renner et al. (1988) found that imidazolinone herbicides were more tightly adsorbed and persisted longer as soil pH decreased. Beckie and McKercher (1989) reported the opposite with a sulfonylurea herbicide, which persisted longer in soils with higher pH levels.

2.4.2 Environmental Conditions

The environment has a large influence on herbicide residue persistence. Many herbicides that have residual activity are degraded in soil by hydrolysis and/or microbial degradation (Beckie and McKercher 1989; Vencill 2002). The relative importance of microbial degradation as compared to chemical hydrolysis is dependant on many factors. Joshi et al. (1985) found that a sulfonylurea herbicide degraded faster in acidic soils because both forms of degradation took place. In alkaline soils however, microbial degradation was the primary source of degradation, resulting in a slower rate of dissipation. Temperature and soil moisture levels have a significant effect on soil microbial populations and activity. Beckie and McKercher (1989) found that lower temperatures and drier soils resulted in the ability to detect herbicide residues with a bioassay for a longer time period after application. Experimental sites, which received a higher level of precipitation, had lower amounts of phytotoxic residues of sulfonylurea herbicides present the year after application (Shinn et al. 1998). Hill et al. (1998) found

that yearly precipitation levels had a significant effect on the persistence of quinclorac, with drier conditions increasing the soil residual half-life of the herbicide. This suggests that in years of lower than average growing season temperatures and/or lower precipitation, residual herbicides may persist longer in the soil. This can have negative effects on sensitive rotational crop species, resulting in reduced yield and/or later maturing crops.

2.4.3 Landscape Position

Renner and Powell (1991) determined that tillage was able to reduce the damage to following crops by residues of soil active herbicides. This was especially true for less mobile herbicides, and may be due to the movement of the residues deeper into the soil profile as a result of plowing. This, along with the incorporation of organic matter may increase microbial activity and decomposition in the soil.

Szmigielska et al. (1998) found slope position had an effect on recoverable sulfonylurea herbicide residues. The greatest degree of herbicide recovery and phytotoxicity occurred on upper slope soils, indicating the highest amount of free herbicide molecules. There was a greater amount of root stunting in soils previously treated with ALS inhibitors compared to untreated in the upper slope soils, as compared to mid slope and lower slope positions. The least amount of root stunting as a percent of the untreated check occurred in lower slope soils, and the least amount of recoverable free sulfonylurea molecules were recovered from these same soils. Schoenau et al. (2005) stated that this increased phytotoxicity of herbicide residues on upper slopes may be due to the higher pH, lower organic matter, and drier conditions that are typical of shoulders and knolls as compared to lower slope positions.

2.5 Bioassay Analysis of Soil-Bound Herbicides

Several bioassays have been developed for the detection of soil residual herbicides. A bioassay involves assessing some component of plant growth such as root length, shoot length, or yield as a function of herbicide concentrations in soil. A bioassay can be used as a quantitative procedure to determine the total amount of a certain herbicide residue present in a soil sample or to assess phytotoxicity (Sunderland

et al. 1991). The application of bioassays to measure ALS inhibiting herbicides in the soil is an effective method as these compounds are potent inhibitors of root and shoot growth of susceptible plants (Brown 1990). This method has proven useful and valid for the detection of several different herbicide residues (Beckie and McKercher 1989; Groves and Foster 1985; Nyffeler et al. 1982; Smigielska et al. 1998; Sunderland et al. 1991). Another major difference between bioassay analysis and analysis by chemical extraction from the soil using an extraction solution is that bioassay analysis is much less expensive, and it can determine the amount of herbicide that is plant available, or phytotoxic, not just the total amount of herbicide present (Groves and Foster 1985). A root length inhibition bioassay is an effective tool to detect small amounts of phytotoxic compounds in the soil, however it may not necessarily reflect yields observed in field.

2.6 Dose-Response Curves

Dose-response curves are used to determine the degree of toxicity a herbicide has on a plant species. This involves adding multiple concentrations of a specific herbicide to the soil and assessing the degree of injury at the different concentrations. An important value derived from the dose-response curve is the I_{50} level. This level represents the concentration of the herbicide that causes 50% injury between the upper and lower asymptote to the test species. The I_{50} value is most commonly used in comparing dose-response curves of the same herbicide in different soils (Onofri 1996).

Moyer and Hamman (2001) used the I_{50} value to determine the effect of soil properties on persistence of herbicide residues, although the term used by those researchers was GR_{50}. The I_{50} value is closely related to soil properties as a measure of herbicide potency because they affect processes like sorption and phytotoxicity (Streibig et al. 1995).

Log-logistic models are the most common models used for bioassay dose-response analysis due to biologically relevant parameters (Hernandez-Sevillano et al. 2001). Seefeldt et al. (1995) recommend the log-logistic model for dose response experiments. Dose-response curves can be compared vertically, in which the same concentrations are evaluated to examine the difference in response, or by horizontal comparison, in which the same response value is evaluated to examine the difference in

concentrations (Streibig 1988). Nielsen et al. (2004) determined that it is possible to compare dose response curves, like those derived from bioassays, separated by time. In controlled environments, there is often very little assay-to-assay variation, allowing for the summarization of time separated bioassays.

2.7 Herbicide Interactions

Interactions among the phytotoxic effects of herbicide compounds have been well documented. Different combinations of herbicides, or other chemicals, can result in either additive, synergistic, or antagonistic interactions. Additive interactions simply mean that there are no interactive effects produced by the combination of the two compounds. Additive effects imply that the herbicides work independently of each other, and the net effect on the desired test species when the herbicides are applied together is the same as the effects of each herbicide applied individually. Synergistic and antagonistic interactions involve interactions that result in significantly more or significantly less toxicity, respectively, to the sensitive species than the sum of the chemical's independent effects (Nash 1981). Colby (1967) developed a mathematical formula that can be used to determine whether or not multiple herbicides applied in combination interact in a synergistic, antagonistic or additive manner. The original intention of this mathematical formula was to determine changes in efficacy when two or more herbicides are combined for control of a weed species. It has also been used to determine changes in plant tolerances to two or more herbicides applied to normally tolerant crop species. It is therefore reasonable to attempt to use this formula to determine the extent to which soil residual herbicides interact when two or more are present in the soil.

3. HERBICIDE INTERACTIONS AS ASSESSED BY LABORATORY DOSE RESPONSE CURVES

3.1 Introduction

Acetolactate synthase inhibiting herbicides, as with all soil residual herbicides, have a range of toxicity to sensitive plant species that depends on application rate in combination with soil properties and environmental conditions. The determination of the phytotoxic range for each soil can be established by creating a dose response curve (Streibig 1988). Dose response curves have been used for a variety of purposes, including the study of persistence and interactions between compounds (Beckie and McKercher 1989; Seefeldt et al. 1995; Webster et al. 2004). A bioassay is one method for creating a herbicide dose-response curve, based upon a range of herbicide rates toxic to a sensitive indicator species.

Dose-response curves have been a valuable tool in determining how varying levels of a compound influence a sensitive species. Weed scientists have been using these curves to determine how a range of herbicide rates affects a target species (Onofri 1996; Ritz et al. 2006). There has also been some work using dose-response curves to examine the persistence of herbicide residues in soil (Nyffeler et al. 1982). Mathematical expressions can be used to determine if the addition of a second herbicide to another, in what is termed a tank mix, can change the level of toxicity (Colby 1967; Nash 1981). One can test for additive, synergistic, or antagonistic interactions between two or more compounds to alter the control of a target species. However, dose response curves as a method to determine the level of interaction of herbicides residing in the soil has not been extensively examined. The objective of this chapter is to present dose response curves developed by using a mustard root length bioassay for five ALS inhibiting herbicides in three contrasting soils, and to assess the nature of the interactions among the compounds.

3.2 Materials and Methods

3.2.1 Laboratory Procedure

Stock solutions of the herbicides to be tested were created by placing a known quantity of herbicide in approximately 50 ml of methanol then diluting with water to the 1L mark in a volumetric flask (Eliason et al. 2004). Standard solutions were created from the stock solution to produce solutions with concentrations of 0.2, 0.4, 0.8, 1.2, 1.6, 2.4 mg a.i. L^{-1} of imazamox/imazethapyr; 10, 20, 30, 40, 60, 80 mg a.i. L^{-1} of imazamethabenz; 0.2, 0.5, 1, 1.5, 2, 3 mg a.i. L^{-1} of flucarbazone-sodium; 0.38, 0.75, 1.13, 1.5, 2.25, 3 mg a.i. L^{-1} of sulfosulfuron; and 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 mg a.i. L^{-1} of florasulam. These concentrations were determined by growing oriental mustard in a variety of herbicide concentrations up to a maximum of two times the recommended field rate and noting what concentration caused the lower asymptote.

Seventy litres of soil (0-10 cm depth) was collected from untreated control plots at each of the Saskatoon, Melfort, and Scott sites where the field trial experiments (see Chapter 4) were run at the same time as the soil sampling of the field trial plots was conducted. The soil was air dried and passed through a 2 mm sieve to remove debris and stones. These soils were then used to perform a root inhibition bioassay to test for herbicide phytotoxicity (Eliason et al. 2004). Oriental mustard (*Brassica juncea* L. 'Cutlass') was selected as the test species for the herbicide root length inhibition bioassay due to its high sensitivity to these herbicides. Mustard seeds were pre-germinated for 24 hours prior to seeding by placing the seeds in a Petri dish on wetted paper towel and placed in the dark at room temperature. One hundred grams of each soil, replicated six times, were placed into Styrofoam® cups. For each cup, 1 ml of the standard solution was added to the untreated soil to produce the concentrations of all five herbicides to reach soil concentrations up to one or two times the recommended field application rate (see Appendix A). This resulted in concentrations of 2, 4, 8, 12, 16, 24 μg kg^{-1} soil of imazamox/imazethapyr; 100, 200, 300, 400, 600, 800 μg kg^{-1} soil of imazamethabenz; 2, 5, 10, 15, 20, 30 μg kg^{-1} soil of flucarbazone-sodium; 3.8, 7.5, 11.3, 15, 22.5, 30 μg kg^{-1} soil of sulfosulfuron; and 0.25, 0.5, 1, 2, 3, 4 μg kg^{-1} soil of florasulam. To test the interactions between herbicides in the lab, 1 ml of each concentration of imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam

Table 3.1: Properties of the soils from the three locations that were utilized for the dose response experiment and field trials.

Soil	% Sand	% Silt	% Clay	O.C.†	pH	F.C.‡
Saskatoon	20	30	50	3.0	7.0	36
Melfort	16	40	44	7.1	6.3	35
Scott	31	42	27	2.7	6.2	24

†percent organic carbon
‡percent moisture (w/w) at field capacity

were utilized in combination with another 1 ml of standard solution of imazamox/ imazethapyr. The imazamox/imazethapyr concentration that caused a reduction of root length to about 70% of the untreated control was utilized for each soil. This concentration was 2 $\mu g\ kg^{-1}$ for the Saskatoon soil, 8 $\mu g\ kg^{-1}$ for the Melfort soil, and 4 $\mu g\ kg^{-1}$ for the Scott soil.

The soil in the cups was then wetted with deionized water to 75% water holding capacity. The soils were then manually mixed to ensure uniform distribution of the added herbicides throughout the soils used for the root inhibition bioassay, and allowed to equilibrate for 24 hours. Five pre-germinated seeds of similar size and radicle protrusion were selected and placed onto the soil surface, covered with a small amount of soil (approximately 0.5 cm) and were lightly packed. The soil was covered with 15 g of high-density polyethylene plastic beads to reduce evaporation losses and wetted to 100% field capacity, placed in a RCBD under a fluorescent canopy (light intensity of 10 $\mu mol\ m^{-2}\ s^{-1}$), and covered with a plastic sheet, which was removed after 24 hours. The plants were watered daily to 100% field capacity by adding deionized water to a pre-determined weight and kept at a constant temperature of 20 °C. On the fifth day after seeding the plants were manually removed from the soil and the root lengths were measured (Figure 3.1). The root length measurements for each plant in the cup were averaged to determine the mean root length. Each of these mean lengths was then converted to percent root length of the untreated check by dividing the mean length of the treated roots by the mean root length of the check and multiplying by one hundred.

3.2.2 Determination of Herbicide Interactions in Soil

In order to determine if the interaction between two different herbicides in the soil is synergistic, additive, or antagonistic, the observed root lengths as a percent of the untreated check values were compared to expected percent root length values predicted by Colby's equation (Colby 1967). The terms in the formula (Equation 3.1) are: E is the expected growth as a percent of the check caused by two combined herbicides, X is the growth as a percent of the check caused by herbicide A, and Y is the growth as a percent of the check caused by herbicide B. By comparing the expected root inhibition (E) to the observed root inhibition, the type of interaction can be discerned. If the observed

Figure 3.1: Oriental mustard plants manually removed from the soil, ready to be measured to determine root length.

percent root length is less than the calculated expected percent root length there is a synergistic interaction. If the observed percent root length is equivalent to the calculated expected percent root length there is an additive interaction. Finally, if the observed percent root length is greater than the calculated expected percent root length the interaction is antagonistic.

$$E = \frac{X\,Y}{100} \qquad [3.1]$$

3.2.3 Statistical Analysis

A log-logistic model in SAS for Windows (Version 9.1, SAS Institute Inc., Cary, N.C., USA) was used to analyze the data with non-linear regression to relate root length to herbicide concentration in the soil (Seefeldt et al. 1995). Equation 3.2 was used to calculate root length as a percent of the untreated check (y) in response to the herbicide concentration added to the soil (x) with parameters for the curve including the upper curve limit (D), the lower curve limit (C), the slope (b), and the concentration that results in a root length which is the mid point, 50%, between the upper and lower asymptotes or limits (I_{50}). This equation creates a sigmoidal curve to best describe fit of the data points with the concentrations of each herbicide. The curve is best described when the data is graphed using a logarithmic scale on the x-axis for the herbicide concentrations because the log-logistic model is used.

$$y = C + \frac{D - C}{1 + (x/I_{50})^b} \qquad [3.2]$$

3.3 Results and Discussion

3.3.1 Bioassay Results from the Laboratory Herbicide Spikes

The dose response curve for the herbicide combination imazamox/imazethapyr (Fig. 3.2) resulted in root length responses that differed among soils at the lower and mid concentration ranges. The root inhibition bioassay produced an equivalent amount of root stunting at higher doses for all three soil types. The root length of the Oriental mustard root inhibition bioassay response to imazamox/imazethapyr varied with soil type. Mustard plants grown in treated Saskatoon soil were affected the most by herbicide concentration in the soil followed by Scott, with herbicide in Melfort soils being the least phytotoxic to the plant as revealed by root length inhibition. The increase in phytotoxicity with increased concentration in the soil was more gradual for the Saskatoon soil than the Scott or Melfort soils.

The dose response curve for imazamethabenz (Fig. 3.3) did not complete the sigmoidal pattern over the range of concentrations used. Phytotoxicity of this compound was substantially reduced in the Melfort soil compared to Saskatoon soil at the higher end of the concentration range. The lower limit of the curve was not reached in any of the soils, even when two times the recommended rate of herbicide was applied to the bioassay samples. The root inhibition observed in the Saskatoon soil showed significantly more stunting compared to the other two soils with imazamethabenz herbicide and stunting occurred at lower concentrations as compared to the other soils. The bioassay as applied to Scott soil resulted in the next most sensitive dose response. This soil required a greater concentration of imazamethabenz to induce measurable root damage, but once this occurred, further increases in concentration resulted in the greatest incremental reductions in root length.

The Saskatoon and Scott soils revealed similar responses to increases in soil concentration for the herbicide flucarbazone-sodium (Fig. 3.4). The Melfort soil has a greater ability to adsorb the herbicide, making it plant unavailable and requiring larger doses to generate phytotoxic responses. As has been observed in previous research (Eliason et al. 2004), organic matter content is a key factor affecting phytotoxicity of this compound via adsorption processes. The Melfort soil has significantly more organic

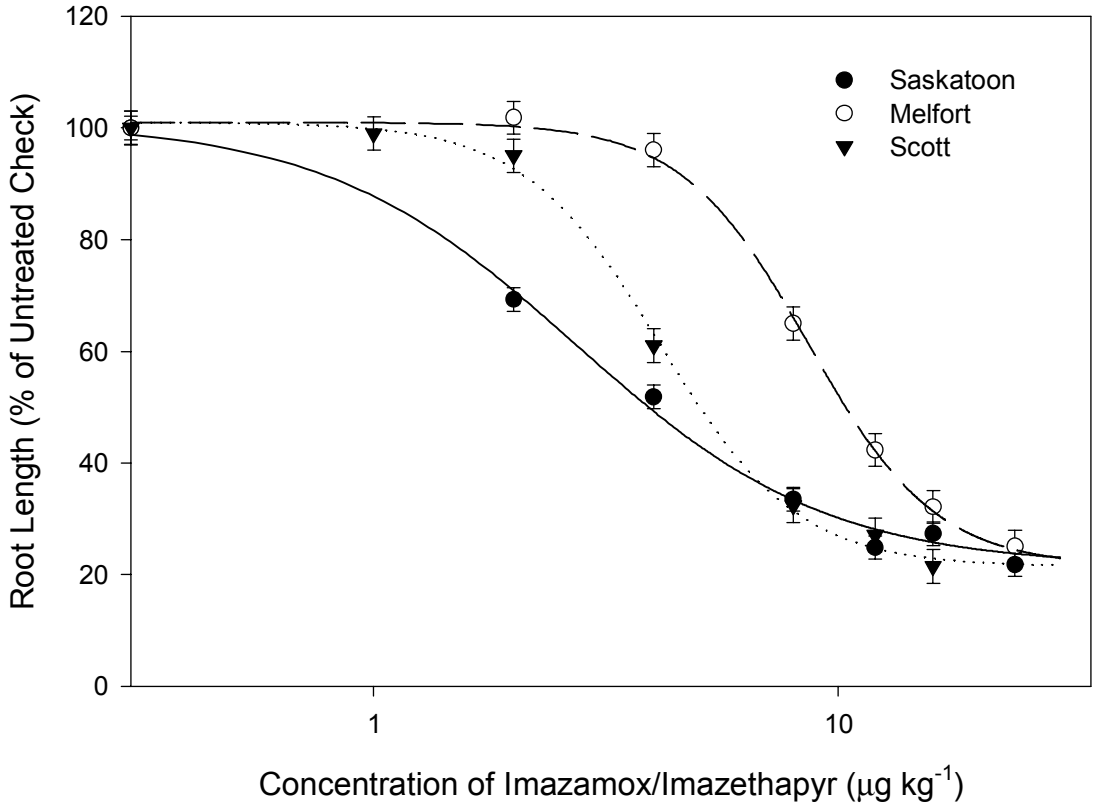

Figure 3.2: Dose response for imazamox/imazethapyr added to three Saskatchewan soils determined by a root inhibition bioassay using Oriental mustard. Each point is the mean of six replicates with bars indicating standard error.

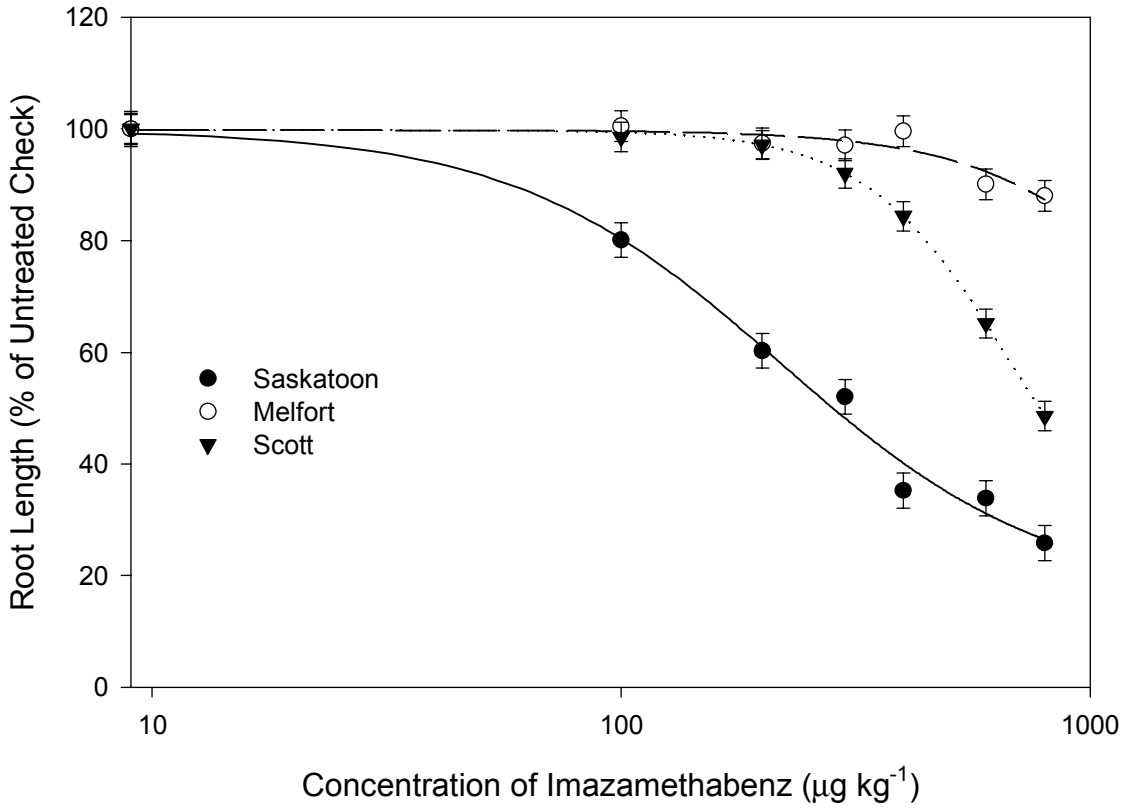

Figure 3.3: Dose response for imazamethabenz added to three Saskatchewan soils determined by a root inhibition bioassay using Oriental mustard. Each point is the mean of six replicates with bars indicating standard error.

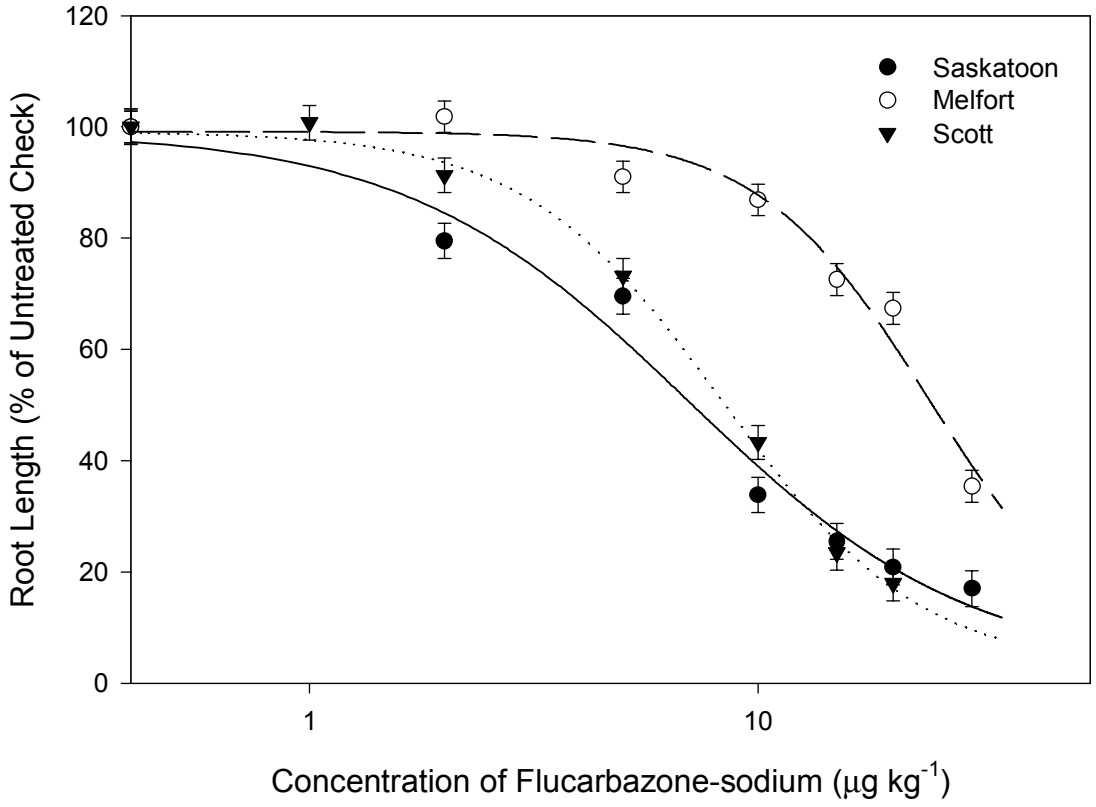

Figure 3.4: Dose response for flucarbazone-sodium added to three Saskatchewan soils determined by a root inhibition bioassay using Oriental mustard. Each point is the mean of six replicates with bars indicating standard error.

matter than the Saskatoon and Scott soils. Therefore, larger doses of the herbicide were required to cause root stunting.

The addition of sulfosulfuron to previously untreated Scott and Melfort soils (Fig. 3.5) resulted in dose response curves with much steeper slopes in the 5 – 10 μg kg^{-1} concentration range than for the Saskatoon soil. A similar pattern to that observed for the other herbicides is evident in the 1 – 10 μg kg^{-1} concentration range, with phytotoxicity following the order Saskatoon > Scott > Melfort.

Dose response curves for florasulam (Fig. 3.6) were similar for Scott and Melfort soils. The shape of the curves for all three soils was similar, and again the phytotoxic effects at a given herbicide concentration were greatest in the order of Saskatoon > Scott > Melfort.

These dose response curves provide an excellent indication of the importance of soil properties in affecting the phytotoxicity of these soil active herbicides. Each of the three soils had the same doses of herbicides applied to the soil 24 hours prior to seeding, and there were large differences in the amount of root stunting, and the dosage level required to initiate root pruning. When equal amounts of herbicides have been applied, there appears to be adsorption primarily to organic matter, with the exception of florasulam, reducing the amount that will be plant available and thus phytotoxic.

By comparing the I_{50} values from the dose-response parameters (Table 3.2), it can be determined if the phytotoxicity of the compound is significantly affected by soil type. There was no difference in I_{50} values between the Saskatoon and Scott soils treated with flucarbazone-sodium. There was a difference between Saskatoon and Scott soils treated with imazamethabenz. For the Melfort soil, a large amount of error in calculating the I_{50} value of imazamethabenz was introduced due to the small amount of root inhibition that was observed, resulting in the need for extrapolation to derive the remaining portion of the curve. Therefore this value was not used for comparisons. All other comparisons of soil types treated with the same herbicide have significantly different I_{50} values, indicating the substantial influence that soil properties have on the plant availability of the herbicides in the soil. By comparing the differences in soil properties between the soils (Table 3.1), it can be deduced that organic matter is perhaps the most important factor affecting the phytotoxicity of ALS inhibiting herbicides in

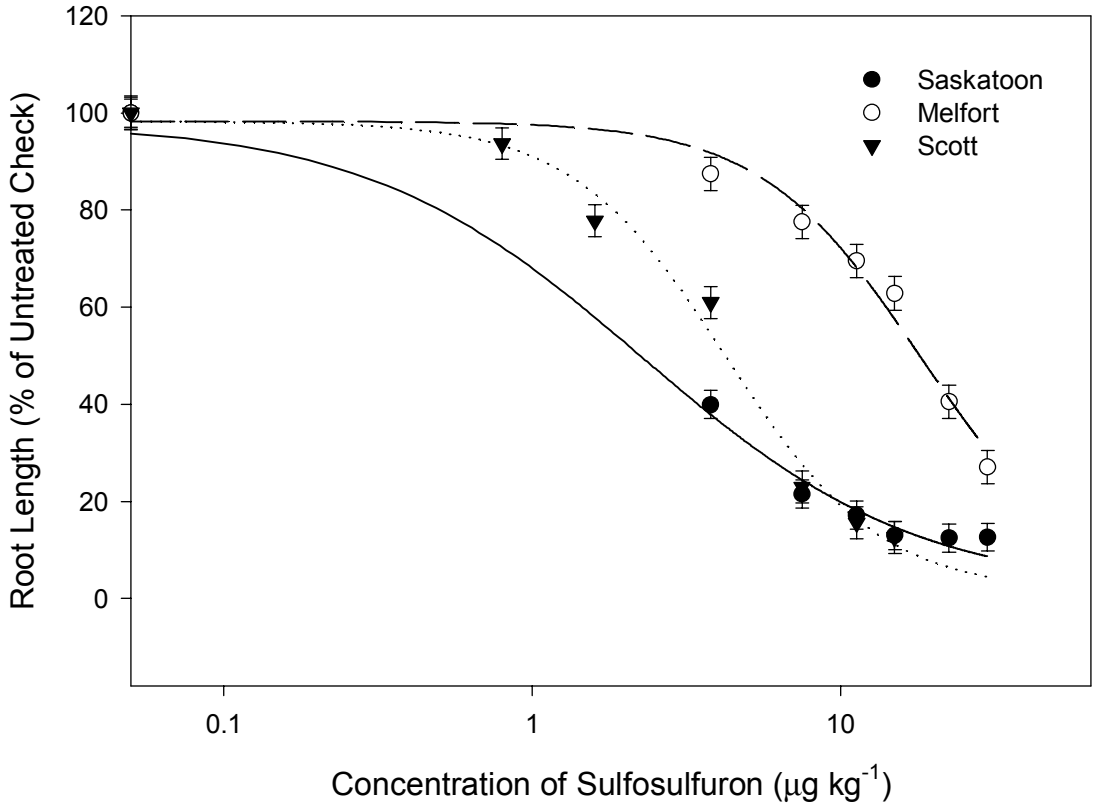

Figure 3.5: Dose response for sulfosulfuron added to three Saskatchewan soils determined by a root inhibition bioassay using Oriental mustard. Each point is the mean of six replicates with bars indicating standard error.

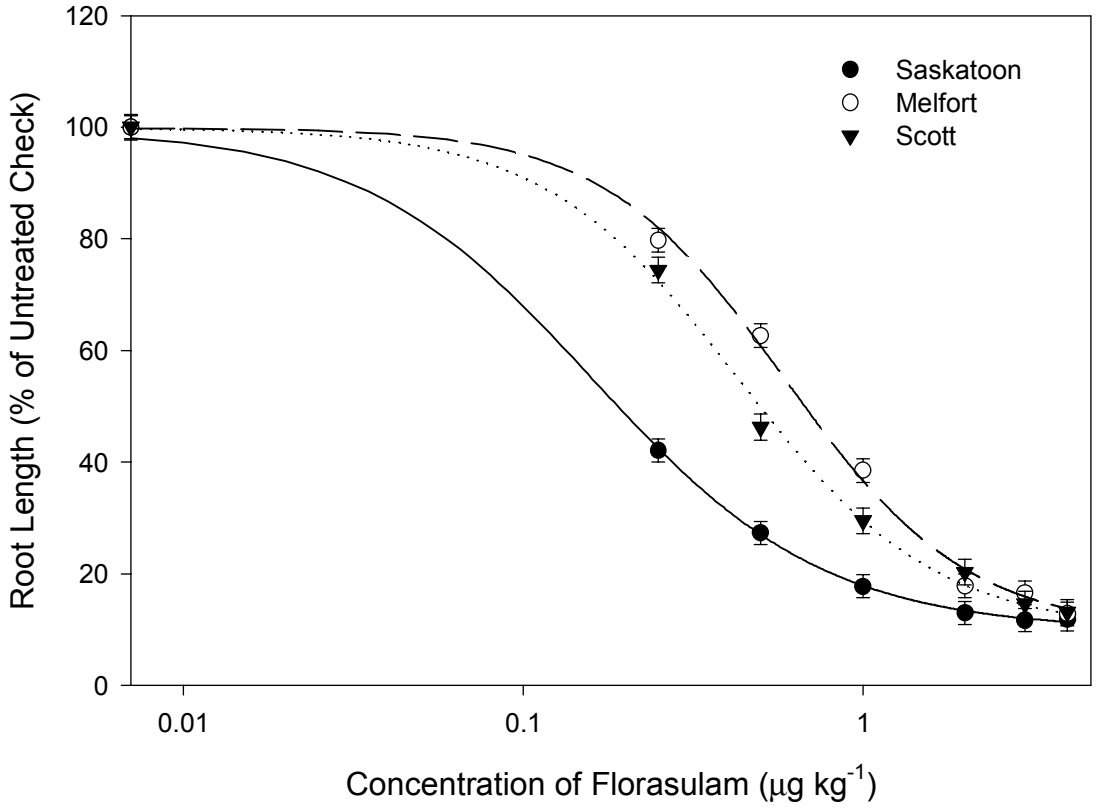

Figure 3.6: Dose response for florasulam added to three Saskatchewan soils determined by a root inhibition bioassay using Oriental mustard. Each point is the mean of six replicates with bars indicating standard error.

Table 3.2: Parameters for the dose response curve for each herbicide applied to soil from each site. The parameters were derived from the non-linear regression of the sample points.

Herbicide	Location	b	C (%)	D (%)	I_{50} (µg kg^{-1})
Imazamox/Imazethapyr	Melfort	3.28	22.64	101.48	8.58 c
	Saskatoon	1.48	18.56	99.89	2.91 a
	Scott	2.86	21.40	100.57	4.16 b
Imazamethabenz	Melfort	-	-	-	-
	Saskatoon	1.50	15.70	99.93	218.01 a
	Scott	2.69	11.70	99.53	702.00 b
Flucarbazone-sodium	Melfort	2.28	0.00	100.82	24.19 b
	Saskatoon	1.72	11.34	97.51	5.83 a
	Scott	1.90	1.66	100.50	8.17 a
Sulfosulfuron	Melfort	1.68	0.00	99.34	18.17 c
	Saskatoon	1.91	11.24	100.00	2.55 a
	Scott	1.97	5.76	98.91	3.80 b
Florasulam	Melfort	1.63	8.84	99.55	0.60 c
	Saskatoon	1.29	9.94	99.99	0.16 a
	Scott	1.51	10.84	100.31	0.41 b

* For a given herbicide, I_{50} values followed by the same letter are not significantly different at $p \leq 0.05$.

soil. Saskatoon and Scott soils have different soil textures and pH with Saskatoon soil having a much higher clay content and higher pH level. However both soils have low organic matter contents compared to Melfort soil, and the high organic matter content in the Melfort soil was likely responsible for greater herbicide adsorption, resulting in less phytotoxicity at similar doses. For all five herbicides, the high clay content of the Saskatoon soil did not appear to be effective in buffering the phytotoxicity of the compounds through adsorption.

3.3.2 Herbicide Interactions

Dose-response curves created with the same herbicides on different soils resulted in curves that were quite different. The amount of herbicide required to cause initial root stunting varied, the slope and shape of the curves tended to vary, and the maximum dose necessary to cause the lower asymptote varied with each soil.

The dose-response curves generated from imazamethabenz applied with imazamox/imazethapyr (Figure 3.7) show differences among the three soils. For the Saskatoon soil there is limited difference between the observed and calculated expected curves. In the Melfort and Scott soils however, there were larger differences. In both cases, at the higher doses there is a trend of more inhibition of the Oriental mustard root length in the bioassay than is predicted by the model, suggesting a synergistic interaction.

Dose-response curves to combinations of flucarbazone-sodium with imazamox/ imazethapyr provide an interesting comparison of observed versus predicted responses (Figure 3.8). As with the previous dose-response curve, the Saskatoon soil the observed and predicted responses are similar. However, the shape of the dose response curves for the Melfort and Scott soils were different, with the model under predicting the amount of root inhibition. This would also suggest a potential synergistic interaction.

The dose-response curves for sulfosulfuron in combination with imazamox/ imazethapyr indicated limited deviation of observed from the calculated expected values, suggesting dominantly additive effects for this combination (Figure 3.9). This agrees with previous work in which Moyer and Hamman (2001) found an additive effect

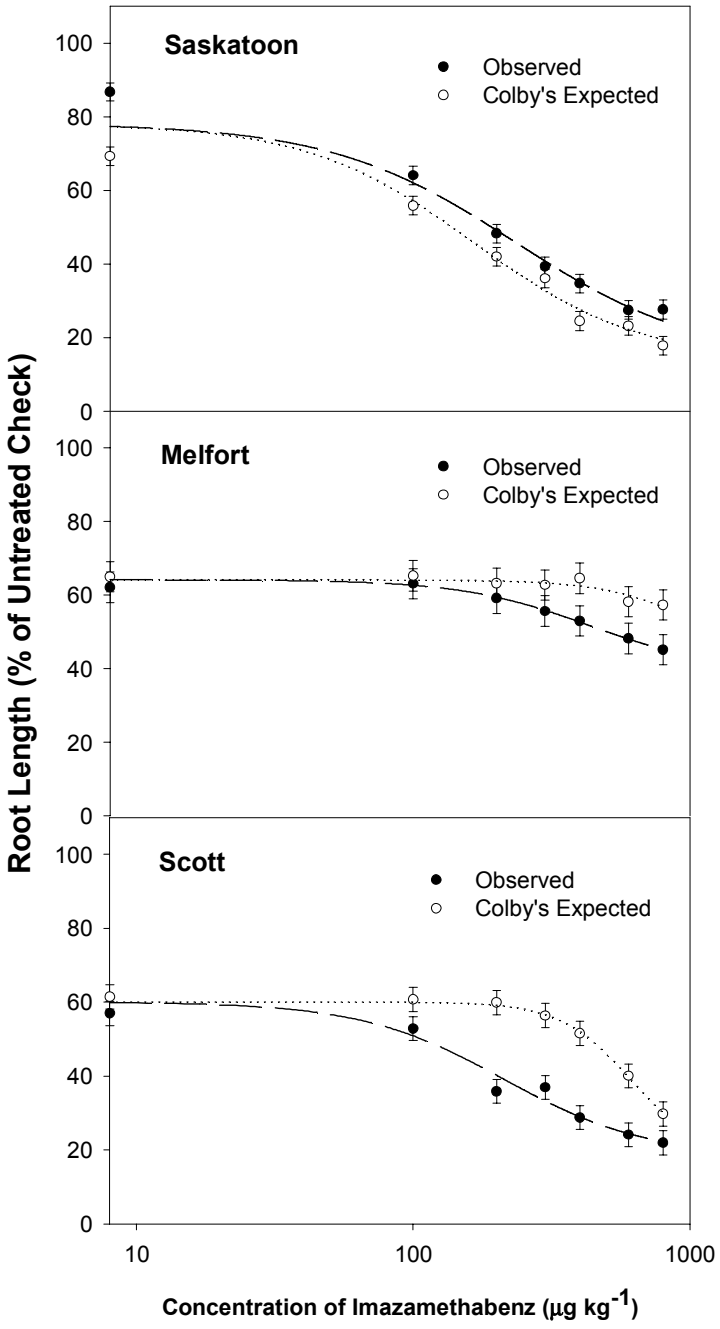

Figure 3.7: Observed and expected root length as a percent of the untreated check to create dose response curves in 3 soils. Values are determined by the root inhibition bioassay using Oriental mustard to increasing concentrations of imazamethabenz in combination with a concentration of imazamox/ imazethapyr that causes approximately 30% root inhibition. This was applied to soil taken from Saskatoon, Melfort, and Scott. Each point indicates mean with standard error bars.

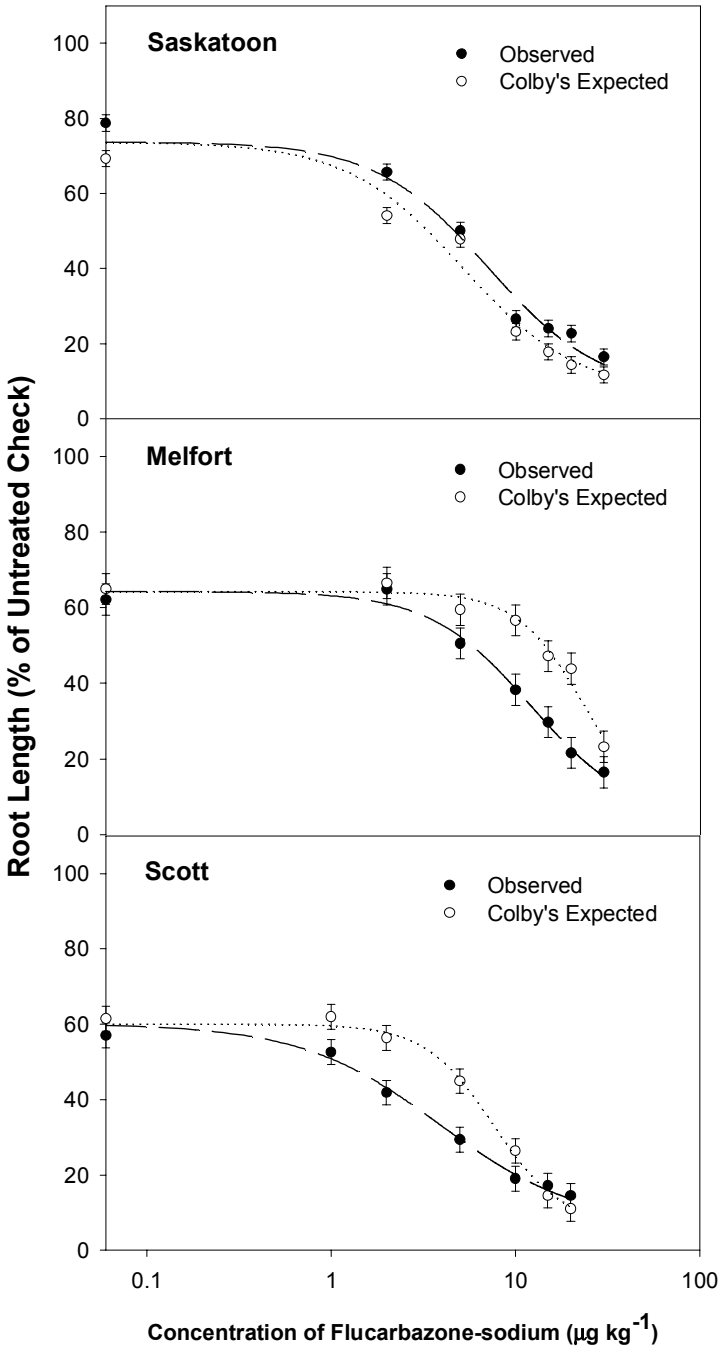

Figure 3.8: Observed and expected root length as a percent of the untreated check to create dose response curves in 3 soils. Values are determined by the root inhibition bioassay using Oriental mustard to increasing concentrations of flucarbazone in combination with a concentration of imazamox/ imazethapyr that causes approximately 30% root inhibition. This was applied to soil taken from Saskatoon, Melfort, and Scott. Each point indicates mean with standard error bars.

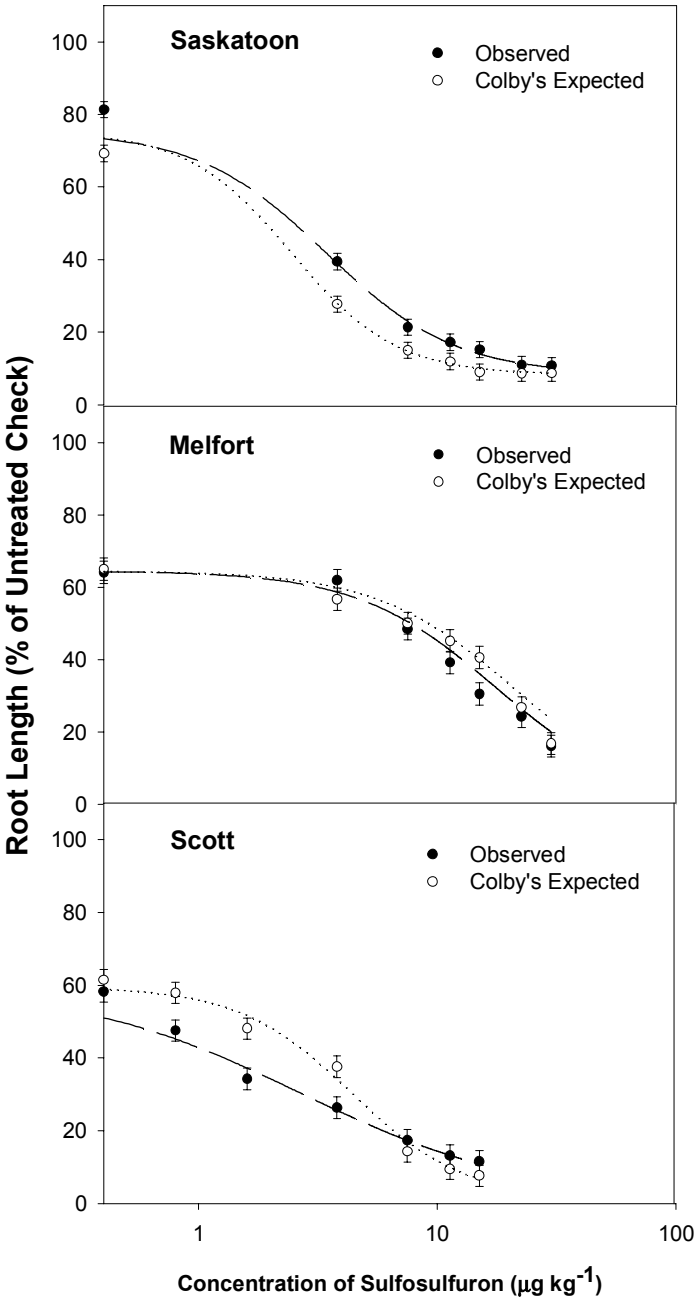

Figure 3.9: Observed and expected root length as a percent of the untreated check to create dose response curves in 3 soils. Values are determined by the root inhibition bioassay using Oriental mustard to increasing concentrations of sulfosulfuron in combination with a concentration of imazamox/ imazethapyr that causes approximately 30% root inhibition. This was applied to soil taken from Saskatoon, Melfort, and Scott. Each point indicates mean with standard error bars.

between sulfosulfuron and other ALS inhibitors applied to soil previously. The Melfort soil dose-response curve did not quite reach its lower asymptote, so the model therefore had to predict that portion of the curve. In the Scott soil the amount of root length inhibition in the bioassay tended to be greater at the lower herbicide doses than predicted by the model.

Combinations of different concentrations of florasulam and imazamox/ imazethapyr produced root length inhibition similar to the calculated expected values (Figure 3.10). Florasulam is highly phytotoxic to mustard, with concentrations less than 1 $\mu g\ kg^{-1}$ responsible for significant reductions in root length. There was a large amount of root pruning at dose levels as low as 0.25 $\mu g\ kg^{-1}$ of florasulam. Therefore it was unreasonable to assume smaller doses could accurately be measured.

One approach to determining if the observed and calculated expected dose response curves are indeed different is to compare the I_{50} values. The parameters that were used in creating the dose-response curves for the observed and calculated Colby's expected data points are shown in Table 3.3. Based on the I_{50} values and using a 95% confidence interval, it could be determined which I_{50} values were significantly different. There was only one combination of herbicides in one of the soils that had a significant difference ($p \leq 0.05$) in I_{50} values. That was the imazamethabenz and imazamox/ imazethapyr combination in the Scott soil. In this case the observed phytotoxicity in the bioassay was much greater than the expected and this suggests that there is the potential in this soil for a synergistic interaction between the herbicides when added together. In all other cases, the lack of significant difference in the I_{50} values indicates that most of the herbicide combinations are behaving in an additive way.

3.4 Conclusion

Soil characteristics have a large influence on the relationship between the concentration of total herbicide in the soil and phytotoxicity according to the Oriental mustard bioassay. The Melfort soil, which is different from the other two soils by high organic matter content, has the potential to adsorb significantly more of the herbicides, explaining the lower phytotoxicity observed at the same doses compared to the Saskatoon and Scott soils. The Saskatoon soil appeared to have less adsorptive

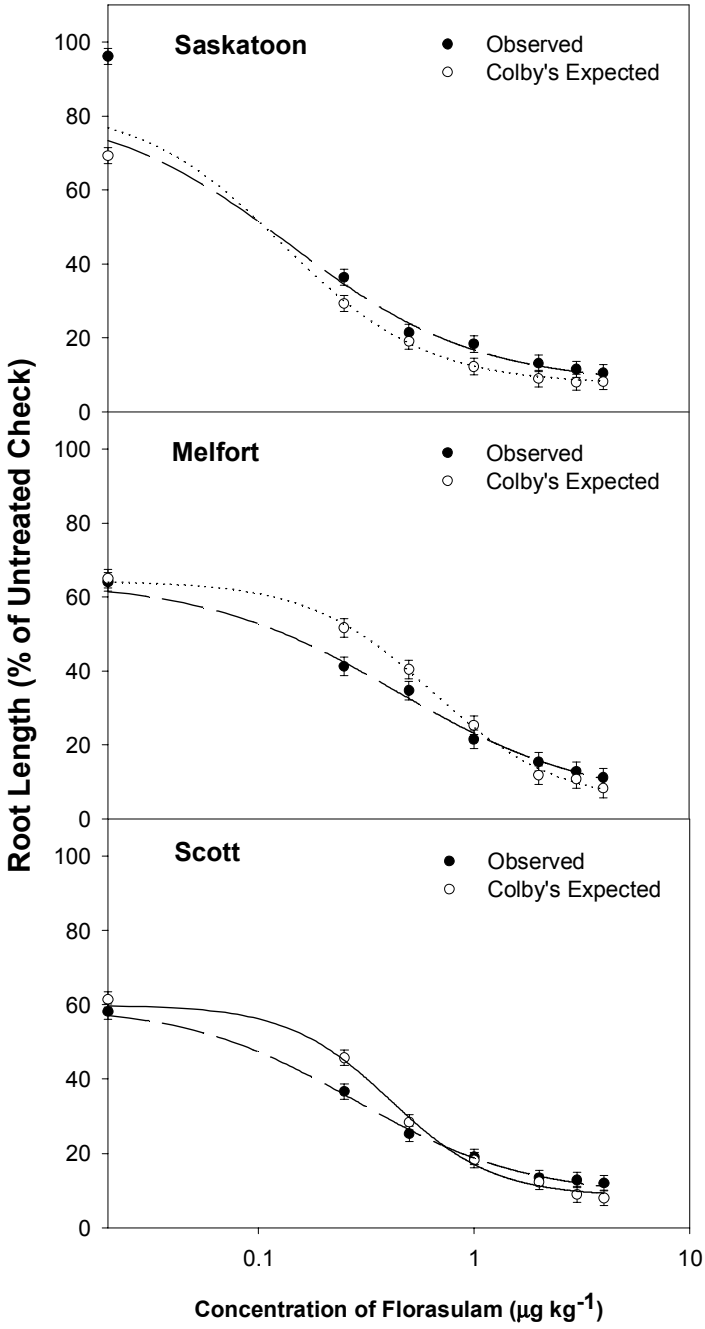

Figure 3.10: Observed and expected root length as a percent of the untreated check to create dose response curves in 3 soils. Values are determined by the root inhibition bioassay using Oriental mustard to increasing concentrations of florasulam in combination with a concentration of imazamox/ imazethapyr that causes approximately 30% root inhibition. This was applied to soil taken from Saskatoon, Melfort, and Scott. Each point indicates mean with standard error bars.

Table 3.3: The parameters for the dose response curves for the observed and the calculated expected values from Colby's equation for each herbicide applied in combination with imazamox/imazethapyr, causing roughly 30% root inhibition, applied to soil from each site. The parameters were derived from the non-linear regression of the sample points.

Herbicide	Location		b	C	D	I_{50}
Imazamethabenz	Saskatoon	Observed	1.29	13.47	78.14	235.2a
		Colby's Expected	1.44	13.47	78.14	165.2a
	Melfort	Observed	1.87	39.23	64.13	-
		Colby's Expected	2.54	39.23	64.13	1121.0a
	Scott	Observed	1.68	18.19	60.04	212.9a
		Colby's Expected	3.40	18.19	60.04	608.5b
Flucarbazone-sodium	Saskatoon	Observed	1.42	6.85	73.71	7.07a
		Colby's Expected	1.39	6.85	73.71	5.10a
	Melfort	Observed	1.59	3.90	64.27	12.08a
		Colby's Expected	2.37	3.90	64.27	23.51a
	Scott	Observed	1.44	18.41	60.03	3.91a
		Colby's Expected	2.33	18.41	60.03	7.58a
Sulfosulfuron	Saskatoon	Observed	1.61	8.34	75.31	3.41a
		Colby's Expected	2.00	8.34	75.31	2.44a
	Melfort	Observed	1.50	0.00	64.49	17.58a
		Colby's Expected	1.51	0.00	64.49	20.73a
	Scott	Observed	0.93	1.26	59.57	2.66a
		Colby's Expected	1.83	1.26	59.57	4.36a
Florasulam	Saskatoon	Observed	1.00	7.57	82.74	0.14a
		Colby's Expected	1.31	7.57	82.74	0.13a
	Melfort	Observed	0.96	4.45	64.36	0.44a
		Colby's Expected	1.49	4.45	64.36	0.64a
	Scott	Observed	1.09	8.46	59.94	0.28a
		Colby's Expected	1.81	8.46	59.94	0.41a

* For a given herbicide at a specific location, I_{50} values followed by the same letter are not significantly different at $p \leq 0.05$.

capability than the Melfort soil, and often the Scott soil, even though the clay content of the Saskatoon soil is higher than the Scott soil. Therefore, immediately after application, organic matter seems to be a major factor involved in removing the herbicide from the soil solution and reducing its phytotoxic effects.

The creation of the model based upon Colby's equation (Colby 1967) and the log-logistic nonlinear regression (Seefeldt et al. 1995) was useful in ascertaining the interaction of soil active herbicides. All combinations, with the exception of imazamethabenz added to the soil with imazamox/imazethapyr in Scott soil, appeared to have simple additive effects. Even with the potential for imazamethabenz added to the soil with imazamox/ imazethapyr for a synergistic interaction, this will likely not be a problem because the herbicides are not recommended for a tank mix application. The I_{50} value was an effective parameter for comparing multiple dose-response curves for common responses to known herbicide concentration applied to the soil.

4. PERSISTENCE AND INTERACTION OF ALS INHIBITING HERBICIDES APPLIED SEQUENTIALLY IN THE FIELD

4.1 Introduction

Residues of some herbicides in the soil can be beneficial during the season of application for control of later flushes of target weeds, thereby reducing potential competition with the crop being grown. However, soil residual herbicides have been found to persist in the soil into the following year, potentially reducing yields of sensitive crops grown in rotation (Cobucci et al. 1998; Johnson et al. 1993; Renner and Powell 1991). For this reason, numerous tools have been developed to predict the relative persistence and risk of damage from these herbicide residues.

Laboratory bioassays are one method that has been developed to detect low concentrations of residual herbicides in soil samples (in the range of 1 part per billion), and tend to be more sensitive and less costly than chemical analysis (Beckie and McKercher 1989; Groves and Foster 1985; Hernandez-Sevillano et al. 2001; Szmigielska et al. 1998). Bioassays have been developed using a variety of plant species and have been successfully used to detect a wide range of concentrations of different herbicides. For example, imazethapyr was detected at 0.5 μg kg^{-1} with beet (Jourdan et al. 1998b), and 0.54 μg kg^{-1} with sugar beet (Bresnahan et al. 2000); flucarbazone-sodium was detected at 1 μg kg^{-1} with Oriental mustard (Eliason et al. 2004); and sulfosulfuron was detected at 1 μg kg^{-1} with sunflower (Hernandez-Sevillano et al. 2001).

The effect of the presence of multiple residues present in the soil due to sequential applications of soil residual herbicides in crop rotations is not well documented. The objective of this study was to determine the persistence and interactive phytotoxic effects of imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam alone and when applied the year after the application of imazamox/ imazethapyr.

4.2 Materials and Methods

4.2.1 Field Trial Setup and Sample Collection

Three locations were selected to represent a range of soil and environmental conditions typically encountered in Saskatchewan: the University of Saskatchewan Kernen Crop Research Farm in Saskatoon (Can.: Dark Brown Chernozem, Sutherland Association; U.S.: Typic Boroll clay loam), and the Agriculture and Agri-Food Canada research stations in Melfort (Can.: Black Chernozem, Melfort Association; U.S.: Udic Haploboroll silty clay) and Scott (Can.: Dark Brown Chernozem, Scott Association; U.S.: Typic Boroll loam). The three Saskatchewan locations provided contrasts in soil properties useful in understanding herbicide persistence (Table 4.1). The experiment was initiated in 2002 and repeated at each location starting in 2003. Environmental conditions at the three sites for the years 2002 – 2004 are shown as growing degree days in Table 4.2 and precipitation in Table 4.3. The experimental design for the field trial was a Randomized Complete Block Design with four replications of ten treatments.

In the first year of the experiment all the plots were seeded to peas (*Pisum sativum* L. 'Swing'), with treatments one through five being sprayed with the non-residual herbicides bentazon and clethodim, and six through ten being sprayed with the residual herbicide mix imazamox/imazethapyr. In year two, all the plots were seeded to wheat (*Triticum aestivum* L. 'Eatonia') with treatments one and six not sprayed with an ALS inhibiting herbicide; two and seven with imazamethabenz; three and eight with flucarbazone-sodium; four and nine with sulfosulfuron; and five and ten with florasulam. All plots seeded with wheat were sprayed with non-residual herbicides clodinafop-propargyl, bromoxynil, and MCPA four days later to control weeds in the check treatments and a maintenance spray for all other plots (see Appendix A for rates). In the third year, all plots were seeded to Roundup Ready™ canola (*Brassica napus* L. 'DKL 3455') and sprayed with glyphosate, a non-residual herbicide. All herbicide rates, expressed in g a.i. ha^{-1}, can be found in Appendix A. In the spring of year 3, before the canola was seeded, three soil samples were taken from every plot with a 10 cm diameter and 7.5 cm long soil coring device. The samples within each plot were combined, but

Table 4.1: Properties of the soils from the three Saskatchewan locations utilized for the field trials and subsequent bioassay analysis.

Soil	% Sand	% Silt	% Clay	O.C.†	pH	F.C.‡
Saskatoon	20	30	50	3.0	7.0	36
Melfort	16	40	44	7.1	6.3	35
Scott	31	42	27	2.7	6.2	24

†percent organic carbon
‡percent moisture (w/w) at field capacity

Table 4.2: Yearly crop growing degree days (base 5°C) at the field trial locations in Saskatchewan (Meteorological Service of Canada, Commercial Weather Services, Saskatoon, SK, Canada).

	Growing Degree Days			
	2002	**2003**	**2004**	**2005**
Saskatoon	1555	1749	1270	1428
Melfort	1451	1656	1175	1268
Scott	1475	1692	1292	1365

Table 4.3: Monthly precipitation (mm) for the four growing seasons at the Saskatchewan field trial locations (Meteorological Service of Canada, Commercial Weather Services, Saskatoon, SK, Canada).

	April	May	June	July	Aug.	Sept.	Oct.	Total
				2002				
Saskatoon	13.1	0.0	73.0	0.0	85.7	59.0	14.5	**286.0**
Melfort	16.7	4.8	56.2	58.0	128.6	42.8	13.4	**387.7**
Scott	3.8	2.5	68.6	31.8	41.8	48.8	17.1	**243.7**
				2003				
Saskatoon	61.2	13.8	30.8	63.9	31.4	38.7	14.0	**292.6**
Melfort	26.2	49.6	52.0	35.8	24.4	23.2	26.2	**274.7**
Scott	24.1	21.8	34.2	66.0	44.6	43.8	14.8	**289.3**
				2004				
Saskatoon	11.8	27.0	79.7	75.0	73.5	21.0	28.9	**402.6**
Melfort	33.2	55.8	81.2	84.9	123.3	34.3	10.0	**536.3**
Scott	2.4	36.5	52.0	58.0	44.6	15.2	14.8	**289.0**
				2005				
Saskatoon	16.0	27.5	160.5	53.5	53.5	74.0	18.0	**523.0**
Melfort	12.8	36.8	165.4	70.0	99.4	97.0	24.5	**600.5**
Scott	27.8	41.4	100.0	76.8	88.6	74.6	14.6	**513.6**

* 30 year long term annual precipitation average (1971 – 2000) for Saskatoon (350 mm), Melfort (412.5 mm), and Scott (358.9 mm).

each treatment and block was kept separate. All samples were kept frozen at –20 ºC until the bioassay analysis could be completed.

4.2.2 Bioassay Analysis of Field Samples

The mustard root length inhibition bioassay described by Eliason et al. (2004) was used to estimate herbicide concentrations in the field trial soils. Once the frozen samples were thawed and air-dried at 35°C, they were passed through a 2 mm sieve. Six replicates of 100 g of soil from each sample was then placed into Styrofoam® cups. De-ionized water was added to the cups to bring the soil moisture content up to 75% field capacity. After 24 hours, five pre-germinated Oriental mustard (*Brassica juncea* L. 'Cutlass') seeds of similar size and radicle protrusion were selected and placed into the Styrofoam® cups, covered with a small amount of soil (approximately 0.5 cm) and were lightly packed. The soil in each cup was covered with 15 g of high-density polyethylene plastic beads to reduce evaporation losses. The cups were wetted to 100% field capacity, placed in a RCBD and under a fluorescent canopy (light intensity of 10 µmol m^{-2} s^{-1}), and covered with a plastic sheet, which was removed after 24 hours. The plants were watered daily with deionized water to 100% field capacity and kept at a constant temperature of 20 °C. On the fifth day after seeding the plants were manually removed from the soil and the root lengths were measured.

The root lengths of each plant in each cup were measured. Each of these mean lengths was then converted to percent root length of the untreated check. By using the dose response curves established for each of the individual herbicides (see Chapter 3), the amount of herbicide that persisted in the field trials into the start of the third experimental year was estimated.

4.2.3 Determination of Soil Herbicide Residue Interactions

Colby's equation (Colby 1967; see equation 3.1) was used to determine if the herbicide residues produced interactions with each other when applied in the field in sequential years. Additive, synergistic, or antagonistic interactions of two herbicide residues were determined by the similarities between the observed bioassay root length values and the expected values calculated with Colby's equation.

4.2.4 Statistical Analysis

The data was analyzed using the mixed procedure in SAS for Windows (Version 9.1, SAS Institute Inc., Cary, N.C., USA) with treatment and location as fixed effects, while year, block, and cup were random effects. The LS means test was used to determine the significant differences between each of the treatment means for a 95% confidence level. These values were used for pre-planned comparisons to determine if the herbicide residues persisted and interacted with previously applied herbicides.

4.3 Results and Discussion

4.3.1 Bioassay Results From the Field Trial Samples

Combined Data

The root length bioassay was used as a tool for determining persistence of the five herbicides in field soil. At each of the three locations (Saskatoon, Melfort, and Scott) different amounts of the herbicides persisted past the season of application according to the root lengths observed in the bioassay. Combining the three sites and both years shows that there can be persistence from residual herbicides one or two years past the season of application (Figure 4.1). For all the treatments of imazamethabenz, flucarbazone, sulfosulfuron, florasulam and the check, the root length inhibition was significantly greater in soil treated previously with imazamox/imazethapyr in year one, than on soil treated with a non-residual herbicide. All the treatments, except flucarbazone and florasulam alone, resulted in significantly lower root lengths than the untreated check. This indicates that small amounts of imazamox/imazethapyr may persist beyond the season of application into the first and second season after application, while sulfosulfuron and imazamethabenz are persisting into the following season after application. When comparing the imazamox/imazethapyr alone to all other treatments, only imazamethabenz and sulfosulfuron treatments combined with imazamox/imazethapyr resulted in significantly shorter root lengths. These results are the same for the previously treated soil as on non-treated soil, indicating flucarbazone-sodium and florasulam are not persisting into the next year, but imazamox/ imazethapyr is.

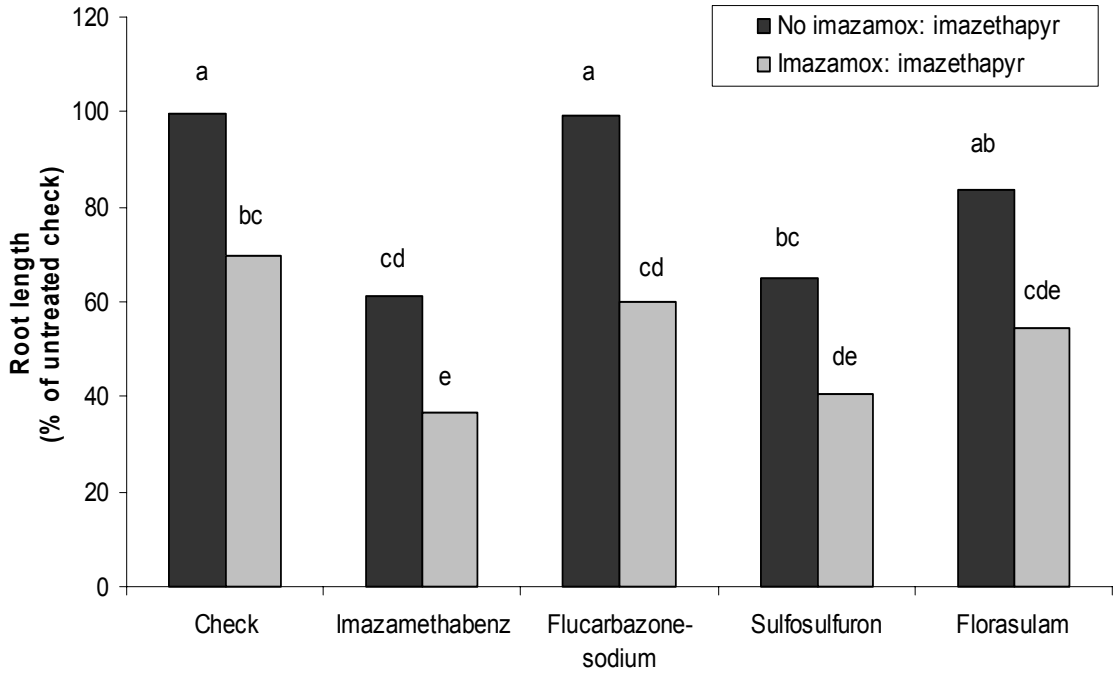

Figure 4.1: Oriental mustard root length responses as a percent of the untreated check averaged over six site years in 3 Saskatchewan soils from samples taken one year after the application of 4 herbicides (if applied) and two years after the application of imazamox/imazethapyr (if applied). Bars with different letters are significantly different with a p value < 0.05.

Saskatoon Site

At the Saskatoon site (Figure 4.2), the percent root length of the mustard bioassay in the flucarbazone and florasulam treatments was not significantly different from the check, indicating that there is no detectable biological activity of these two compounds in this soil. The treatment that had only an application of imazamox/imazethapyr in the first year, with a non-residual herbicide the next year, had a lower mean root length but was not significantly different than the check, thus indicating that at this site imazamox/ imazethapyr did not persist two years after application. However, both imazamethabenz and sulfosulfuron treated soils had significantly lower root lengths from the untreated check indicating that these two compounds persisted into the following season. The root lengths of the mustard bioassays grown in soil treated with imazamox/imazethapyr alone was not significantly different from the root lengths in soil treated with imazamox/ imazethapyr and imazamethabenz or sulfosulfuron. This again indicates that imazamox/ imazethapyr is not persisting two seasons after application at the Saskatoon site. However, when flucarbazone and florasulam were applied to soil already treated with imazamox/imazethapyr, the root lengths from the bioassay are significantly less than the check, indicating damage. These three herbicides alone do not show persistence in the root length inhibition bioassay, but when flucarbazone or florasulam is applied to soil previously treated with imazamox/imazethapyr there can be enough phytotoxic residue to cause root stunting in a sensitive species. The root stunting is only observed when these herbicides are combined, not when applied alone, indicating that in this soil it requires the combination of imazamox/imazethapyr with either flucarbazone or florasulam to cause detectable residual damage. This is likely due to an interaction of the herbicides either additively or synergistically, which is examined later in section 4.3.2.

Melfort Site

At the Melfort site (Figure 4.3), florasulam was the only herbicide that persisted beyond the application season as evident by the significantly lower root length compared to the check while all other wheat herbicides resulted in statistically equivalent root

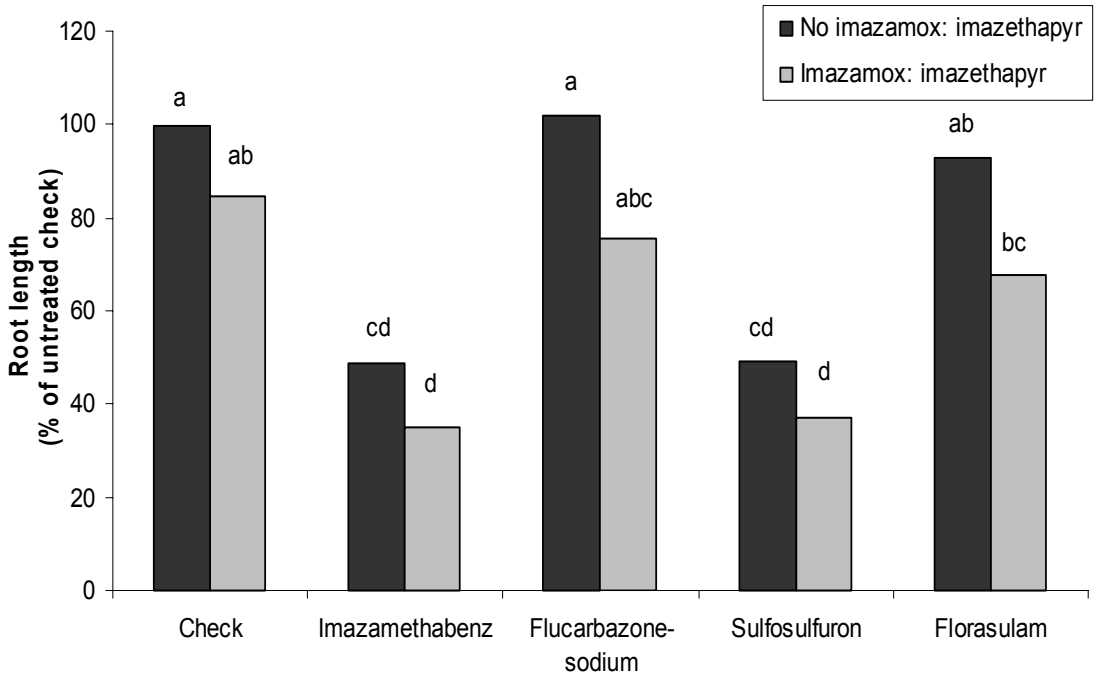

Figure 4.2: Oriental mustard root length responses as a percent of the untreated check averaged over two site years in Saskatoon soil from samples taken one year after the application of 4 herbicides (if applied) and two years after the application of imazamox/imazethapyr (if applied). Bars with different letters are significantly different with a p value < 0.05.

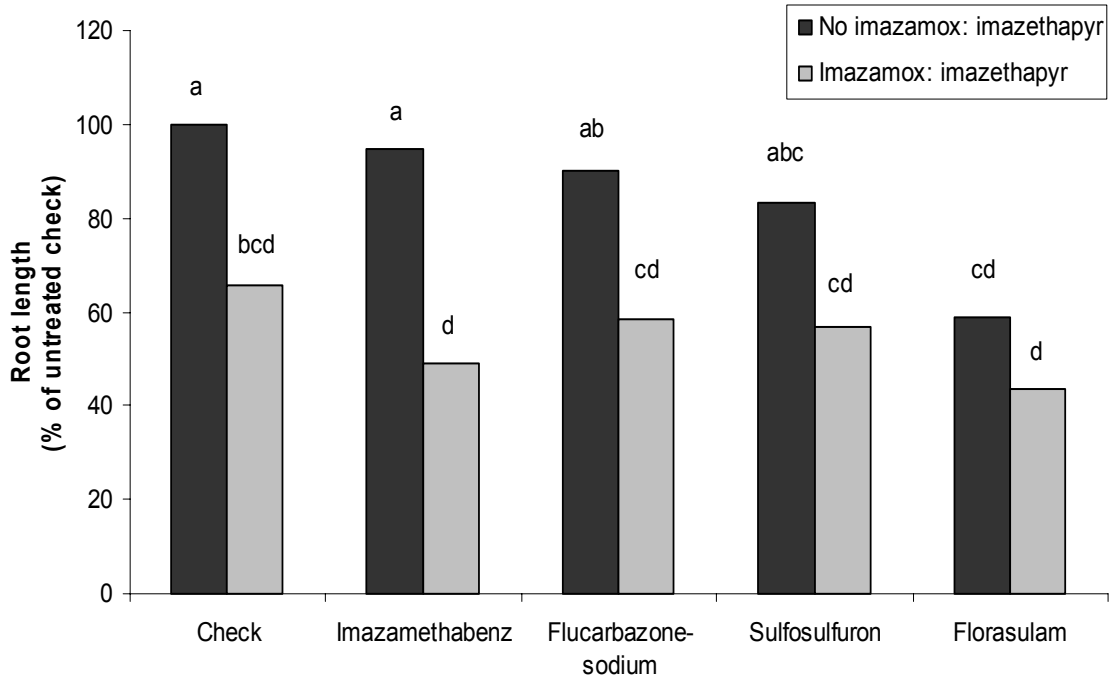

Figure 4.3: Oriental mustard root length responses as a percent of the untreated check averaged over two site years in Melfort soil from samples taken one year after the application of 4 herbicides (if applied) and two years after the application of imazamox/imazethapyr (if applied). Bars with different letters are significantly different with a p value < 0.05.

lengths. Imazamox/imazethapyr persisted two seasons after application, resulting in significant root stunting with all imazamox/imazethapyr treatments compared to the untreated check. Florasulam again was the only herbicide that did not result in a significant difference between imazamox/imazethapyr treated and untreated soil, and also was not significantly different from the application of imazamox/imazethapyr alone. This is interesting because florasulam persisted, and imazamox/imazethapyr persisted yet together there was no increase in root damage. This indicates a possible interaction between the herbicides. The interaction appears to potentially be antagonistic because the addition of the two herbicides caused less damage than expected. Application of Colby's test for the type of interaction is covered in the next section. All other treatments involving combinations of two herbicides produced similar root lengths from the bioassay as compared to imazamox/imazethapyr alone. This indicates that the majority of the damage in these trials was, in fact, due to the persistence of imazamox/ imazethapyr.

Scott Site

Results from the root length inhibition bioassay applied to the Scott soil samples (Figure 4.4) yielded results similar to the Saskatoon site. With the exception of flucarbazone and florasulam alone, all the herbicides significantly reduced the mustard root lengths as compared to the check, indicating persistence. Imazamox/imazethapyr, sulfosulfuron, and imazamethabenz are evidently very persistent at the Scott site, with imazamox/imazethapyr residues causing significant bioassay damage two seasons after field application. Imazamethabenz was the only herbicide not to cause significantly more root damage when combined with imazamox/imazethapyr. Imazamethabenz resulted in the most root stunting, possibly reducing the impact of the imazamox/ imazethapyr on the root length inhibition bioassay. Flucarbazone and florasulam added to soil previously treated with imazamox/imazethapyr resulted in root lengths that were not significantly different from imazamox/imazethapyr alone. This also indicates that this damage was caused by imazamox/imazethapyr, not flucarbazone or florasulam.

The difference in results observed at different locations may be attributed to differences in soil properties (Table 4.1) as well as weather conditions (Table 4.2 and 4.3). Soils with higher organic matter and clay contents will adsorb more of the

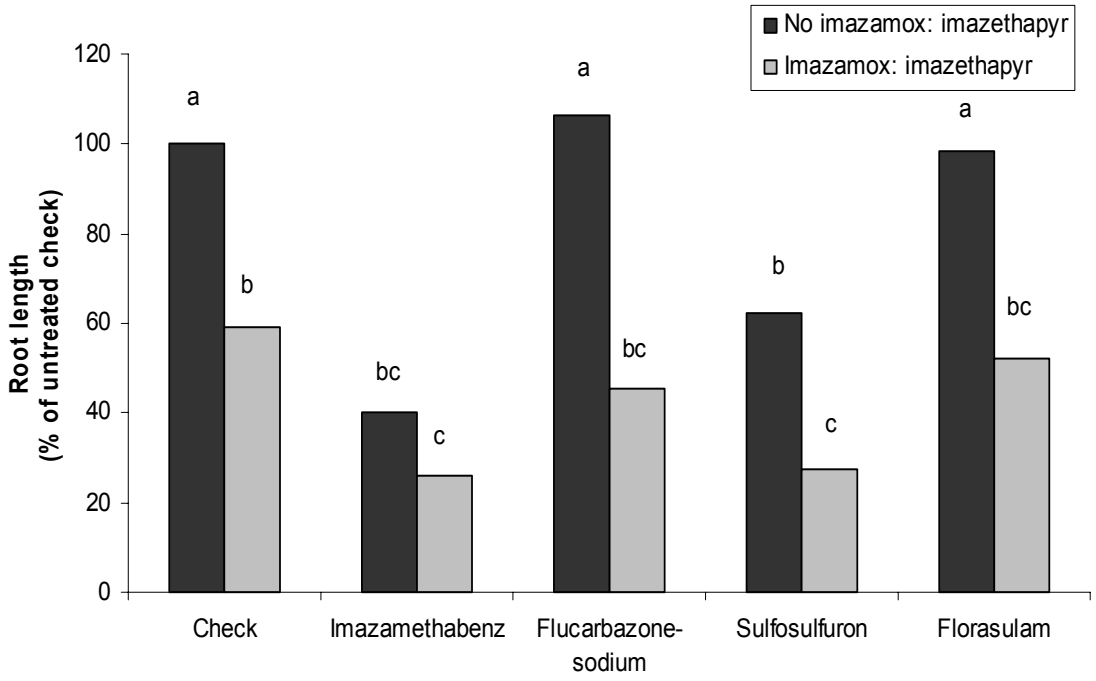

Figure 4.4: Oriental mustard root length responses as a percent of the untreated check averaged over two site years in Scott soil from samples taken one year after the application of 4 herbicides (if applied) and two years after the application of imazamox/imazethapyr (if applied). Bars with different letters are significantly different with a p value < 0.05.

imidazolinone herbicides, making less available for plant uptake (Bresnahan et al., 2000; Moyer and Hamman, 2001). It can be observed from the weather data that it was hotter with more growing degree days, and drier with less precipitation at each of these locations for the first two years (2002-2003) of this experiment compared to the latter two years (2004-2005). Increased soil temperature, which is directly influenced by ambient temperature, is associated with faster degradation rates of residual herbicides (Beckie and McKercher, 1989; Helling, 2005; Shaner and Hornford, 2005).

Soil moisture levels, which vary with yearly precipitation in dryland agriculture, also directly influence persistence. Increasing soil moisture will decrease the length of detection period of soil residual herbicides with the exception of soil saturation that, unless anaerobic degradation occurs, will extend the persistence period (Beckie and McKercher, 1989; Goetz et al., 1990; Helling, 2005). This change in weather may have had an impact on the results obtained from the two repeats of the experiment. In the first experiment the residues were subject to degradation under the hot, dry conditions of 2002 and 2003, while the repeat of the experiment involved imazamox/imazethapyr also subject to degradation under hot, dry conditions of 2003 while the four wheat herbicides were subjected to cooler, wet conditions of 2004. Due to the variability of these years, statistically the year portion of the data was treated as a random effect. Location, however, as illustrated in Table 4.4 resulted in no treatment effect. There was, however, a treatment by location effect because some herbicides persisted at one location and not others due to differences in soil characteristics and environmental conditions.

4.3.2 Determination of Residue Interactions

In order to determine how the herbicide residues were interacting with each other in the soil, Colby's equation (Equation 3.1) was applied to the data to determine expected percent root lengths (Colby 1967). These calculated values were compared to the actual observed results from the root inhibition bioassay. There was no significant difference at a 95% or 90% confidence level between the observed root lengths and the expected root lengths as a percentage of the control calculated for the combined sites (Figure 4.5), or for the Saskatoon (Figure 4.6), Melfort (Figure 4.7), or Scott (Figure 4.8) sites individually. This indicates that there was an additive interaction in all cases.

Table 4.4: Statistical analysis of the fixed effects for determination of significance level for the field experiment.

	DF	F value	P value
Treatment	9	9.87	0.0011
Location	2	0.3	0.7697
Treatment*Location	16	3.31	0.0100

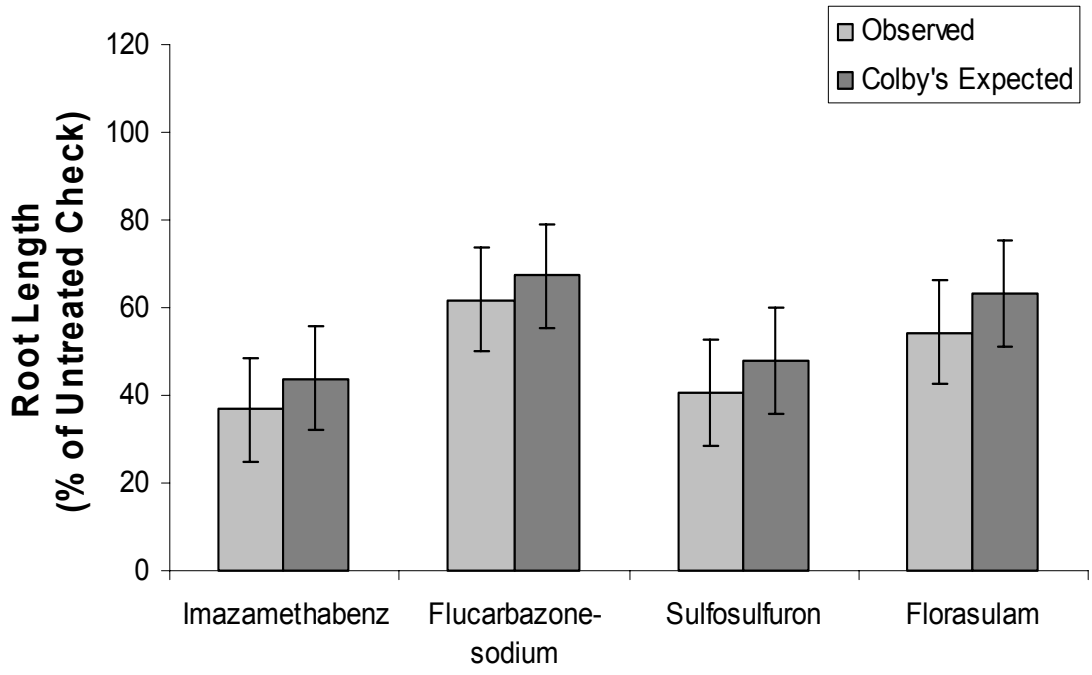

Figure 4.5: Root length as a percent of the untreated check derived from the root inhibition bioassay for the four herbicide treatments in combination with imazamox/imazethapyr for all sites combined over two years versus the expected values derived from Colby's equation (Colby 1967). Each bar represents mean with standard error.

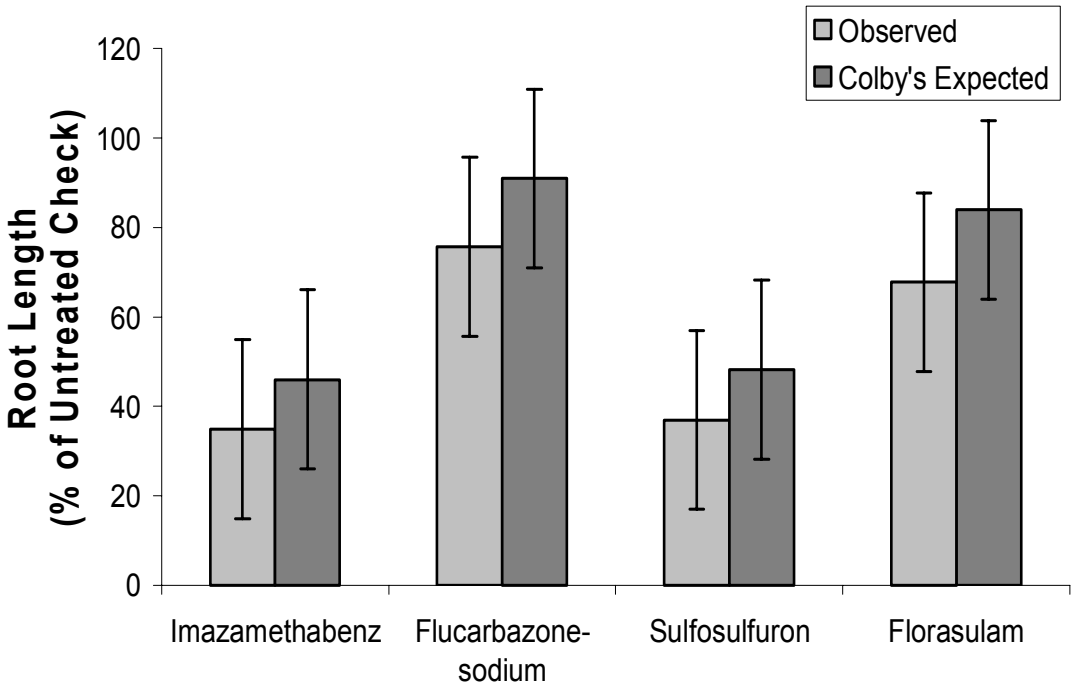

Figure 4.6: Root length as a percent of the untreated check derived from the root inhibition bioassay for the four herbicide treatments in combination with imazamox/imazethapyr for the Saskatoon site combined over two years versus the expected values derived from Colby's equation (Colby 1967). Each bar represents mean with standard error.

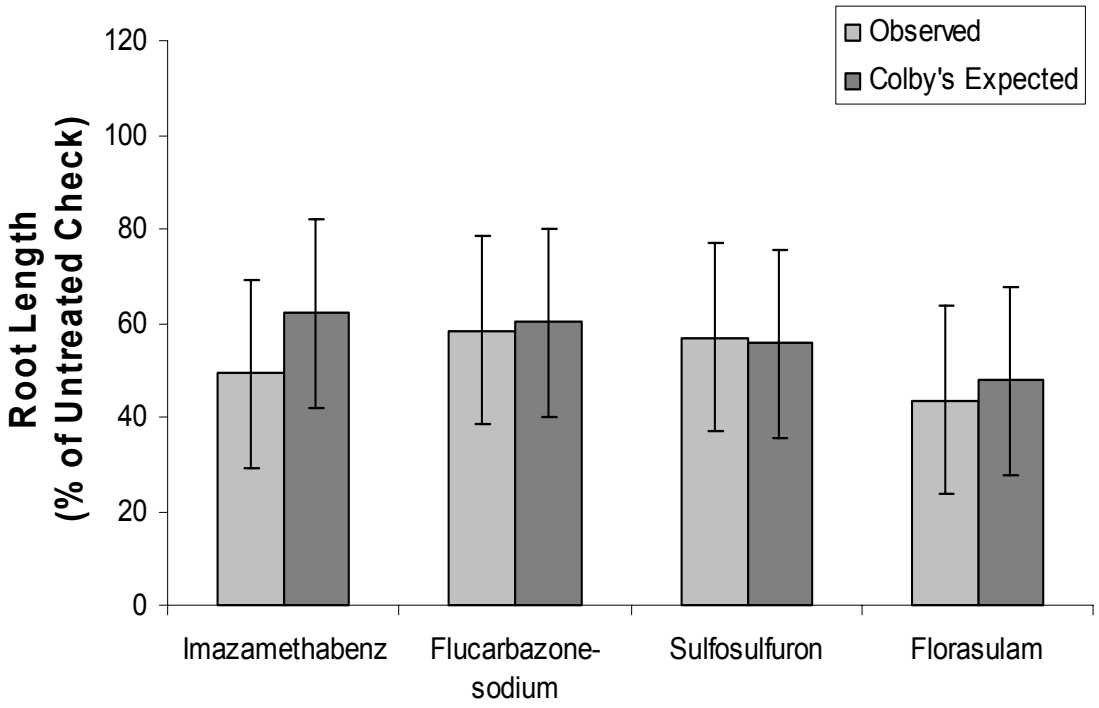

Figure 4.7: Root length as a percent of the untreated check derived from the root inhibition bioassay for the four herbicide treatments in combination with imazamox/imazethapyr for the Melfort site combined over two years versus the expected values derived from Colby's equation (Colby 1967). Each bar represents mean with standard error.

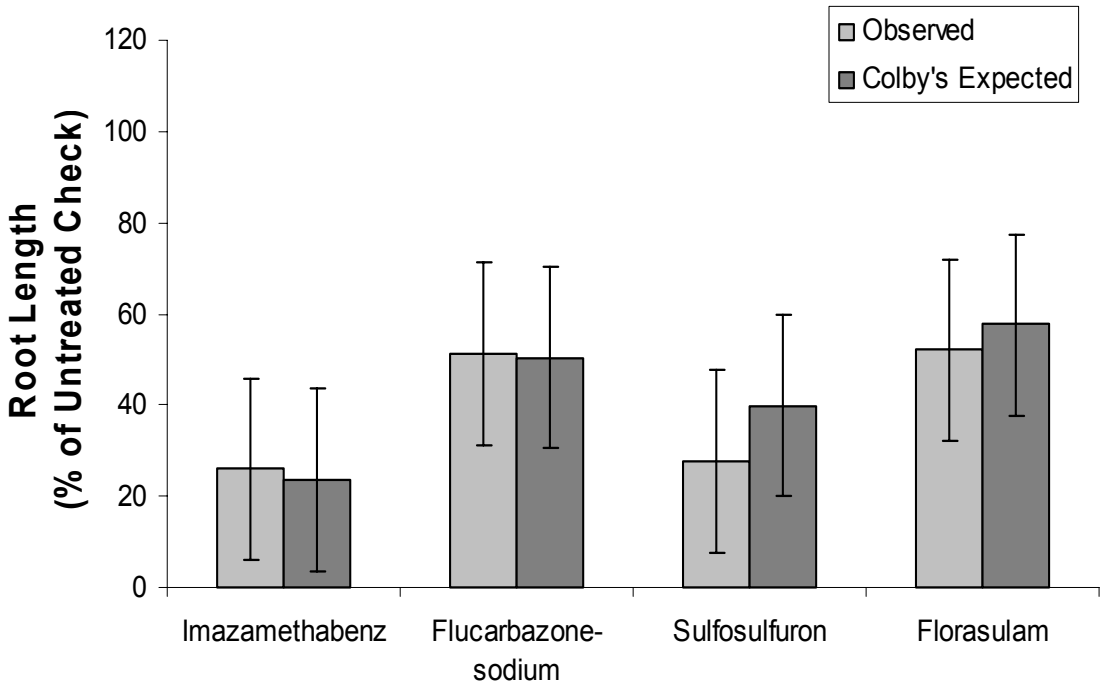

Figure 4.8: Root length as a percent of the untreated check derived from the root inhibition bioassay for the four herbicide treatments in combination with imazamox/imazethapyr for the Scott site combined over two years versus the expected values derived from Colby's equation (Colby 1967). Each bar represents mean with standard error.

According to the application of Colby's equation, there is no indication that any of the questionable results were, in fact, synergistic or antagonistic residue interactions. This means that the residues each contribute to reduction of the root length independently of each other. As the damage is manifested on the same sensitive root, the resulting stunting is worse than when either of the two residues is present alone.

It was noted previously that in the Saskatoon soil, flucarbazone and florasulam treatments when combined with imazamox/imazethapyr caused some detectable root length reduction, which did not appear when imazamox/imazethapyr was not present. None of these herbicides caused root length inhibition individually, but together there was some persistence and phytotoxic effect. According to the results generated from Colby's equation (Fig. 4.6), there is no interaction, rather the damage caused by the residues is not detectable individually, but becomes detectable when added together. Florasulam and imazamox/imazethapyr were noted to have a possible antagonistic interaction in the Melfort soil (Fig. 4.7). However, based upon the results derived by using Colby's equation, when comparing the observed with the calculated expected percent root lengths, it can be seen that there is no significant difference between the two values.

It can be observed in Figures 4.5, 4.6, 4.7, and 4.8 that there are slight differences between the calculated expected values and the observed bioassay values. For these small differences, the observed value is typically slightly lower than the calculated expected value. This suggests that the calculation is slightly underestimating the damage caused to the actual roots by the residual herbicides. However, there is a large amount of variation with each of the Oriental mustard bioassay root lengths. This large amount of variation reduces the accuracy in making deductions based upon small differences.

4.3.3 Quantification of Herbicide Residues

The root length reduction measurements observed in the bioassays were used to determine whether or not herbicides persisted in a soil. However, in the treatments where a single herbicide was applied alone, it is possible to estimate how much of the herbicide is still in the soil at the time of testing using a calibration curve. The mean

bioassay root length for each herbicide concentration in the soil at each location was determined using the addition of known concentrations of herbicides as described in Chapter 3. Using the dose response curves established (Figures 3.1 through 3.5) it is possible to estimate residue concentration using the dose response curves as calibration curves (Beckie and McKercher, 1989). The estimated concentrations are presented in Table 4.5. The concentrations that are estimated from root lengths that are statistically shorter than the untreated check are marked with asterisks. The values indicate the amount of the herbicide left in the soil that would be plant available one year after the previous seasons application, except for imazamox/imazethapyr, which had two years between the season of application and year of sampling.

One of the potential problems encountered with the estimation of herbicide residue concentration in soil that has been in the field for a period of time based on root length inhibition determined by fresh applications of herbicide to the soil may be seen with imazamethabenz from the Scott site. According to estimations derived from the dose response curve, to achieve the same extent of stunting of roots that occurred in field samples as determined by the dose response curve indicates a concentration level that would result from over two times the application rate based on the herbicide remaining in the top 10 cm. This indicates that either there was an error in application of herbicide to the field treatment, an error in creating the dose response curve, or that in a low pH, low O.M., light textured soil, imazamethabenz residue becomes more phytotoxic over time than when it is immediately applied.

4.4 Conclusion

With the exception of flucarbazone-sodium, all the residual ALS inhibiting herbicides were found to persist past the season of application to varying degrees. Each of these remaining concentrations resulted in variable root stunting depending on soil type and environment, reflecting the extent to which soil properties influence the phytotoxic portion of the total amount of residue present. In the case of imazamox/imazethapyr, the residues persisted two years past the season of application to produce phytotoxic effects in the bioassay. The greatest amount of root stunting evident from the root length inhibition bioassays occurred when imazamox/imazethapyr was

Table 4.5: Estimated amount of residual herbicide present in the soil based on the Oriental mustard root inhibition bioassay compared to dose response curves derived from laboratory addition of herbicides.

	Amount of Residual Herbicide (μg kg^{-1} soil)		
	Saskatoon	Melfort	Scott
Imazamox/imazethapyr	1.1	8.1*	4.3*
Imazamethabenz	292*	143	919*
Flucarbazone-sodium	0.0	9.5	0.0
Sulfosulfuron	3.0*	6.8	3.1*
Florasulam	0.0	0.5*	0.0

* indicates root lengths significantly lower ($p < 0.05$) than the untreated check.

present in the soil with another residual herbicide. However, the effects of the two compounds appeared to produce an additive injury effect. No synergistic or antagonistic interactions were observed as determined by Colby's equation. There was generally a great deal of root stunting with imazamox/imazethapyr combined with imazamethabenz and sulfosulfuron. Caution should be used when recropping to sensitive species after these herbicides have been utilized in successive years on the same field.

5. SUMMARY AND CONCLUSION

An Oriental mustard root length inhibition bioassay was used to assess the phytotoxicity, persistence and interactions of five ALS inhibiting herbicides in three prairie soils. The bioassay method was found to be a simple and sensitive tool in detecting small amounts of herbicides present in the soil.

The dose response modeling of the ALS inhibitors imazamox/imazethapyr, imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam indicated that soil properties greatly influenced the phytotoxicity of the applied herbicides. The Melfort soil with its high organic matter content, over two times that of the Scott and Saskatoon soils, appeared able to adsorb the compounds to the greatest extent. This reduced phytotoxicity, which resulted in higher concentrations of herbicides being required to induce the equivalent amount of root stunting as compared to the other two soils. Organic matter content appears to be an important soil property that should be considered when attempting to predict the phytotoxicity and persistence of ALS inhibiting herbicides in prairie soils (Eliason et al. 2004; Moyer and Hamman 2001). The organic matter content appears to be one of the main factors controlling ability of prairie soils to adsorb ALS inhibiting herbicides and rendering them less plant available. In the case of florasulam, soil pH may also play a role, with more florasulam sorbed at lower pH as in the Scott soil.

The Oriental mustard root inhibition bioassay was an effective tool to detect herbicide residue carry over in the field one or two years past the season of application. Imazamox/imazethapyr was still being detected by the bioassay in the soil samples taken from the Melfort and Scott field trials almost two years after the herbicide was applied. Flucarbazone-sodium was the only herbicide that did not persist into the next growing season. Florasulam was also less likely to persist at phytotoxic levels, or cause significant root stunting in the bioassay. Imazamethabenz and sulfosulfuron were the most phytotoxic the following year according to the bioassay, often resulting in significant root stunting.

An important finding of this study is that there was no evidence of any antagonistic or synergistic interactions of the herbicide residues in field trials at these three Saskatchewan locations. The injury from sequential field applications of ALS inhibiting herbicides over two years was additive in nature. However, this implies that the potential is still there for greater phytotoxic effects when two separate residual herbicides are applied as compared to only one. This was observed at the Saskatoon location where no phytotoxic residues of imazamox/imazethapyr, flucarbazone-sodium, and florasulam were detectable with the Oriental mustard root length bioassay. However, when flucarbazone and florasulam were applied to plots one year after the application of imazamox/imazethapyr, the bioassay indicated injury through root stunting. This illustrates the potential for rotational crops to be injured from two residues present and acting together in an additive manner as compared to only one herbicide residue present.

Based on the results of this experiment for three Saskatchewan soils, it appears that soil organic matter is possibly the most important factor affecting the phytotoxic soil residues of imazamox/imazethapyr, imazamethabenz, flucarbazone, sulfosulfuron, and florasulam. In conjunction with soil organic matter, soil pH also appeared to have an influence upon the adsorptive abilities of a soil. Surprisingly, clay content in the soil did not appear to influence the phytotoxicity of herbicide residues as much as expected. However the overall effect of these three soil properties requires further study with a larger number of soils to clearly reveal the relative importance of each factor for an individual herbicide. In addition to the soil property factors which influence phytotoxicity, weather conditions including moisture levels and temperature can influence injury due to persistence. Reduced soil moisture levels and ambient temperatures can reduce the rate of herbicide degradation in the soil by microbes or hydrolysis. In addition to slowed degradation, these conditions can also put stress on the plant species increasing the likelihood of injury.

The single most important recommendation that can be made is that the sequential application of residual ALS inhibiting herbicides over two years should be avoided if sensitive crop species are grown in rotation. Even though there is little evidence of synergistic or antagonistic interactions, the potential injury due to the

additive effects is still present. If there is no alternative, soils with high organic matter levels appear to effectively buffer residue injury to roots, and ample soil moisture appears to aid in degradation of the residues, resulting in less injury potential.

Future work towards a better understanding of ALS inhibitor persistence and interaction of residues is recommended to include bioassay analysis of a wide range of soils in which the effect of a single property like organic matter, pH, or texture can be factored out. This could be achieved by altering pH levels or organic matter contents of soils to determine the effect. The nature and degree to which newly applied compounds versus aged compounds and their metabolites affect phytotoxicity, persistence and interactions in soil also deserve further attention.

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APPENDICES

Appendix A: Calculation for Herbicide Concentration in Soil

1×10^9 cm^3 ha^{-1} based upon herbicide remaining in top 10cm of soil

Application rates applied to soil based upon soil bulk density of 1.3 g cm^{-3}

$$\frac{x \text{ g a.i. herbicide}}{\text{ha}} \times \frac{\text{ha}}{1 \times 10^9 \text{ cm}^3} \times \frac{1 \times 10^6 \text{ µg}}{\text{g}} \times \frac{\text{cm}^3}{0.0013 \text{ kg}} = x \text{ µg kg}^{-1} \text{ soil}$$

	Herbicide Field Application Rates	
Herbicide	**g a.i. ha^{-1}**	**µg kg^{-1} soil**
Imazamox/imazethapyr	30	23
Imazamethabenz	502	386
Flucarbazone-sodium	29	22
Sulfosulfuron	21	16
Florasulam	5	4

Year	**Herbicide Over-spray**	**Herbicide Field Application Rates g a.i. ha^{-1}**
1	Bentazon	840
	Clethodim	89
2	Clodinafop-propargyl	56
	Bromoxynil	280
	MCPA	280
3	Glyphosate	450

Appendix B1: Mean Oriental Mustard Root Lengths for All Dose Response Treatments

		Bioassay Root Length (cm)					
	Rate	**Saskatoon**		**Melfort**		**Scott**	
Treatment	($\mu g\ g^{-1}$ soil)	Ind.	Com.	Ind.	Com.	Ind.	Com.
Imazamox/ Imazethapyr	0	5.14		7.55		7.82	
	1					7.72	
	2	3.54		7.64		7.41	
	4	2.64		7.21		4.77	
	8	1.71		4.86		2.54	
	12	1.27		3.20		2.11	
	16	1.39		2.42		1.67	
	24	1.11		1.88			
Imazamethabenz	0	5.05	4.72	8.00	4.77	7.98	4.80
	100	4.03	3.48	8.03	4.84	7.86	4.47
	200	3.00	2.63	7.78	4.54	7.75	3.02
	300	2.62	2.14	7.75	4.28	7.33	3.11
	400	1.75	1.89	7.95	4.09	6.73	2.43
	600	1.69	1.49	7.18	3.72	5.18	2.04
	800	1.29	1.50	7.04	3.48	3.87	1.85
Flucarbazone	0	5.59	4.28	7.96	4.77	8.32	4.80
	1					8.34	4.41
	2	4.39	3.57	8.06	5.01	7.55	3.55
	5	3.86	2.72	7.23	3.88	6.03	2.47
	10	1.86	1.44	6.90	2.97	3.55	1.61
	15	1.43	1.31	5.77	2.28	1.95	1.45
	20	1.16	1.24	5.34	1.68	1.47	1.23
	30	0.94	0.90	2.82	1.27		
Sulfosulfuron	0	5.75	4.42	8.24	5.22	8.04	4.64
	0.8					7.49	3.79
	1.6					6.20	2.72
	3.8	2.29	2.15	7.19	5.04	4.94	2.09
	7.5	1.23	1.16	6.39	3.95	1.78	1.38
	11.3	0.98	0.94	5.72	3.19	1.24	1.05
	15	0.75	0.83	5.17	2.48	1.01	0.92
	22.5	0.72	0.60	3.34	1.98		
	30	0.73	0.59	2.21	1.31		
Florasulam	0	5.66	5.23	7.60	5.22	8.23	4.64
	0.25	2.37	1.98	6.04	3.36	6.08	2.91
	0.5	1.52	1.17	4.75	2.83	3.80	2.01
	1	1.00	1.00	2.96	1.75	2.42	1.51
	2	0.74	0.72	1.35	1.26	1.67	1.07
	3	0.66	0.63	1.25	1.05	1.20	1.01
	4	0.67	0.58	0.97	0.91	1.07	0.95

Ind. – Individual application; Com. – Combined with Imazamox/Imazethapyr.

Appendix B2: Mean Oriental Mustard Root Lengths for All Field Trial Treatments

Treatment	Location	Bioassay Root Length (cm)
Untreated Check	Saskatoon	6.24
	Melfort	7.54
	Scott	8.89
Imazamethabenz	Saskatoon	3.02
	Melfort	7.11
	Scott	3.60
Flucarbazone	Saskatoon	6.13
	Melfort	6.73
	Scott	9.39
Sulfosulfuron	Saskatoon	2.72
	Melfort	6.24
	Scott	5.51
Florasulam	Saskatoon	5.63
	Melfort	4.19
	Scott	8.74
Imazamox/Imazethapyr	Saskatoon	4.89
	Melfort	4.75
	Scott	5.22
Imazamox/Imazethapyr and Imazamethabenz	Saskatoon	2.14
	Melfort	3.54
	Scott	2.30
Imazamox/Imazethapyr and Flucarbazone	Saskatoon	4.45
	Melfort	4.16
	Scott	4.07
Imazamox/Imazethapyr and Sulfosulfuron	Saskatoon	1.82
	Melfort	4.23
	Scott	2.43
Imazamox/Imazethapyr and Florasulam	Saskatoon	4.23
	Melfort	3.12
	Scott	4.61

Appendix C: Nutrient Concentration for the Three Experimental Soils

	Soil Nutrient Content (µg g^{-1} soil)			
	NO_3	NH_4	P	K
Saskatoon	37.8	5.4	62.29	999.3
Melfort	159.8	8.4	89.44	958.0
Scott	26.8	2.4	108.73	908.3