Soil Properties and Agri-Environmental Conditions Affect Imazamox: Imazethapyr (1:1) and Flucarbazone-Sodium Phytotoxicity and Dissipation

by

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A Thesis submitted to the Faculty of Graduate Studies of

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Soil Properties and Agri-Environmental Conditions Affect Imazamox: Imazethapyr (1:1) and Flucarbazone-Sodium Phytotoxicity and Dissipation

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ABSTRACT

Halabicki, Paula S. M.Sc., The University of Manitoba, December 2009. Soil Properties and Agri-Environmental Conditions Affect Imazamox:Imazethapyr (1:1) and Flucarbazone-Sodium Phytotoxicity and Dissipation. Major Professor: Annemieke Farenhorst.

In 2002, approximately one out of four farmers in Manitoba used a herbicide product containing the combined active ingredients imazamox and imazethapyr. The active ingredient flucarbazone-sodium is equally popular, with 29 % of producers surveyed (2002) in Manitoba using herbicide products containing this active ingredient. Imazamox, imazethapyr and flucarbazone-sodium, classified as Group 2 (ALS inhibitor) herbicides, are relatively persistent in soil (with reported half-lives of 20-30, 60-90 and 17 days, respectively), and hence herbicide residues may damage subsequent sensitive crops when herbicide residues persist and are bioavailable to the plant by root uptake. In addition, herbicide residues may persist into years when other Group 2 herbicides are applied. In 2002, 37 % of Manitoba respondents surveyed applied soil residual ALS inhibitors in successive years. Concerns have been raised about these repeated applications after field agronomists reported increased incidence of field pea injury when fields were treated with imazamox:imazethapyr (1:1) following flucarbazone-sodium applications in the previous year.

No published research was found on the phytotoxicity of imazamox:imazethapyr (1:1) in Manitoba soils, and only one study was found for flucarbazone-sodium phytotoxicity in Manitoba soils. This M.Sc. project utilized an oriental mustard root

bioassay applied to four Manitoba soils to determine the impact of soil properties, nitrogen applications, herbicide co-applications, soil moisture conditions and soil temperature on herbicide dissipation, particularly phytotoxicity. Root length, as a percent of control, was the response measured in the bioassay that has been shown an effective indicator of flucarbazone-sodium phytotoxicity.

Results of the phytotoxicity experiments described in Chapters 2 and 3 demonstrated that oriental mustard was generally more sensitive to imazamox: imazethapyr (1:1) than to flucarbazone-sodium residues in soil. For both herbicides, phytotoxicity showed an inverse correlation with soil organic carbon content, suggesting that herbicide sorption by soil decreased the bioavailability of herbicide residues to plants. Quantification of the sorption of imazamox and imazethapyr by each of the four soils confirmed this, as a negative correlation between sorption and phytotoxicity was observed. The effect of nitrogen on herbicide phytotoxicity was dependent on soil characteristics, the concentration of nitrogen applied, and the concentration of herbicide applied. The effects of herbicide co-application were additive or synergistic (i.e. stacking) or antagonistic depending on soil characteristics and the amounts of herbicide residues in soil.

For the dissipation experiments described in Chapter 4, soils were incubated with herbicides at a range of moisture contents (50, 75 or 100 % field capacity), a range of temperatures (5, 15 or 25°C), or a range of soil nitrogen concentrations (0, 75 or 150 kg N ha^{-1}). Results indicated that the phytotoxicity throughout incubation of both imazamox:imazethapyr (1:1) and flucarbazone-sodium was smallest at 100 % field capacity and at 25°C and that herbicide phytotoxicity increased with decreasing soil

moisture contents or soil temperatures because of the lesser herbicide degradation in drier and cooler soils. Soil moisture had a greater effect on the dissipation of imazamox: imazethapyr (1:1), while root length response in flucarbazone-sodium-treated soils was more affected by declining temperature. Effects of soil nitrogen treatments on herbicide dissipation were minimal for flucarbazone-sodium, but pronounced for imazamox: imazethapyr (1:1), where phytotoxicity increased with increasing soil nitrogen level, suggesting that the addition of nitrogen to soil increases herbicide sensitivity.

This research supports the notion that weed control or crop injury is not determined by the total chemical concentration of the herbicide in soil, but by the bioavailability of the herbicide residues to the plant. As demonstrated, herbicide bioavailability and hence phytotoxicity is influenced by many factors, some of which interact. In order to minimize the potential for crop damage following the use of imazamox:imazethapyr (1:1) or flucarbazone-sodium, well-planned rotations must be devised, particularly for soils that are of coarse texture, with low organic carbon contents and that are dry and cool throughout the growing season.

FORWARD

This thesis was prepared in the manuscript format in accordance with the Department of Soil Science guidelines.

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1. INTRODUCTION

Imazamox:imazethapyr (1:1) and flucarbazone-sodium, categorized as Group 2 herbicides, are frequently used in western Canadian agriculture. They are classified according to their mode of action, which is the inhibition of ALS (acetolactate synthase) (also referred to as AHAS (acetohydroxyacid synthase)), a major enzyme in the biosynthesis of valine, leucine, and isoleucine amino acids (Miflin, 1971).

The active ingredients imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxy-methyl)-3-pyridinecarboxylic acid) and imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5ethyl-3-pyridine-carboxylic acid) are imidazolinones most commonly used for the post emergence control of both grassy and broadleaf weeds in field peas, soybeans, fenugreek, alfalfa and imidazolinone-tolerant (Clearfield) canola and lentil (Anonymous, 2009a; Vencill, 2002a; Vencill, 2002b). Imazamox:imazethapyr (1:1) is marketed by BASF Canada under the label name Odyssey® (35 % imazethapyr, 35 % imazamox), and is also a component of Absolute® (35 % imazethapyr, 35 % imazamox, 360 g/L clopyralid). In 2002, approximately one-quarter of producers surveyed in Manitoba used herbicide products containing these combined active ingredients (Leeson et al., 2002).

Flucarbazone-sodium (1H-1,2,4-triazole-carboxamide,4,5-dihydro-3-methoxy-4-methyl5-oxo-N-[[2-(trifluoromethoxy)phenyl] sulfonyl]-sodium salt), a sulfonylamino-carbonyltriazolinone, is a relatively new post emergence herbicide used to control grassy

and some broadleaf weeds in spring wheat and durum (Anonymous, 2009b; Vencill, 2002c). Fucarbazone-sodium is marketed by Arysta LifeScience Canada under the label name Everest® (66 % flucarbazone). In western Canadian agriculture, it is most frequently used in Manitoba where, in 2002, nearly one-third of producers surveyed used herbicide products containing this active ingredient (Leeson et al., 2002).

Group 2 herbicides, including active ingredients imazethapyr, imazamox and flucarbazone-sodium, have a strong potential to persist in soil past the season of application, potentially damaging subsequent sensitive crops (Jourdan et al., 1998b; Loux et al., 1989; Moyer and Esau, 1996; O'Sullivan et al., 1998). Herbicide residues in soil can be phytotoxic when they are bioavailable to the plant by root uptake, and this herbicide bioavailability is influenced by soil chemical and physical properties (Eliason et al., 2004; Ortega et al., 2004; Williams et al., 2002).

Bioassays are sensitive, simple techniques that can measure bioavailable herbicide residues in soil and aid in understanding the relation between soil properties and herbicide phytotoxicity and dissipation over time. Eliason et al. (2004) tested five crops and determined that oriental mustard (*Brassica juncea*) root length was the best indicator for quantifying the bioavailable concentrations of flucarbazone-sodium residues in soil.

Eliason et al. (2004) measured flucarbazone-sodium phytotoxicity and persistence in five Saskatchewan soils ranging in texture from sandy loam to clay with organic carbon contents of 1.1 to 3.8 % and one Manitoba heavy clay soil with 4.3 % organic carbon content. They found that flucarbazone-sodium phytotoxicity was much greater in five Saskatchewan soils as compared to the Manitoba soil, but that its soil half-life was significantly greater in the Manitoba soil. There have been no published studies on

imazamox:imazethapyr (1:1) phytotoxicity and persistence in Manitoba soils; however, elsewhere, imazamox and imazethapyr have been found to persist longer in soils with increased clay and organic matter contents due to increased sorption (Ahmad et al., 2001; Goetz et al., 1990; Loux et al., 1989). Thus the use of imazamox:imazethapyr (1:1) and flucarbazone-sodium on Manitoba soils with higher clay and organic matter contents needs to be examined.

The observed increased persistence of flucarbazone-sodium in Manitoban soils as compared to Saskatchewan soils (Eliason et al., 2004), suggests the greater potential for Manitoba soils to contain flucarbazone-sodium residues when other ALS inhibitor (Group 2) herbicides are applied to the same soil. Johnson et al. (2005) reported increasing frequencies of "back-to-back" ALS inhibitor usage on the Prairies, and in 2002, Leeson et al. (2002) found that 37 % of Manitoba respondents to their Weed Survey Questionnaire applied soil residual ALS inhibitors in successive years. In 2001, the Weed Subcouncil of the Saskatchewan Advisory Council on Soils and Agronomy raised concerns about these repeated applications after field agronomists reported increased incidence of field pea (*Pisum sativum* L.) injury when fields were treated with imazamox:imazethapyr (1:1) following flucarbazone-sodium applications in the previous year (Johnson et al., 2005). The repeated use of residual herbicides resulting in either additive or synergistic phytotoxicity to rotational crops, has been termed herbicide residue "*stacking*", as defined by Johnson et al. (2005). Johnson and other researchers are currently conducting field and laboratory studies with Saskatchewan and Alberta soils to investigate the potential risk associated with ALS inhibitor stacking (Geisel et al., 2008). To date, little work is being conducted using Manitoba soils.

The persistence of herbicides in soil can be affected by agri-environmental factors such as soil moisture content, soil temperature and nutrient application, through the effect of these factors on herbicide sorption and degradation rates. Generally, increasing soil moisture contents (to field capacity) and increasing soil temperatures (to 30°C) enhance microbial activity and hence herbicide degradation, but these factors have less of an effect on herbicide sorption and desorption processes (Aichele and Penner, 2005; Anderson, 1984; Eliason et al., 2004; Flint and Witt, 1997; Gaultier et al., 2009; Goetz et al., 1990; Jenkins et al., 2000; Jourdan et al., 1998a; Zimdahl et al., 1984). Nitrogen, in various formulations, makes up the largest segment (55 % in 2004) of fertilizer sales in Manitoba (Anonymous, 2004). Soil nitrogen levels have been shown to influence the susceptibility of plants to herbicides, however results differ based on the plant species and herbicide applied (Cathcart et al., 2004; Chao et al., 1994; Lutman et al., 1975). No work has been conducted on the influence of soil nitrogen applications on the phytotoxicity or dissipation of flucarbazone-sodium or imazamox:imazethapyr (1:1) herbicides.

The goal of this project was to improve the understanding of the persistence and bioavailability of imazamox:imazethapyr (1:1) and flucarbazone-sodium in Manitoba soils. This goal was divided into three separate studies, which each had a specific objective.

Study 1 – Soil Properties Affect Imazamox:Imazethapyr (1:1) and Flucarbazone-Sodium Phytotoxicity. The objective of study one was to use the oriental mustard root bioassay (Eliason et al., 2004) to quantify the effect of soil properties and ammonium

nitrate application, on the phytotoxicity of imazamox:imazethapyr (1:1) and flucarbazone-sodium in Manitoba soils.

Study 2 – Imazamox:Imazethapyr (1:1) and Flucarbazone-Sodium "Stacking" in Manitoba Soils. The objective of study two was to quantify the interaction responses of imazamox:imazethapyr (1:1) and flucarbazone-sodium applied to four southern Manitoba soils using the oriental mustard root bioassay.

Study 3 – Dissipation of Imazamox:Imazethapyr (1:1) and Flucarbazone-Sodium in Manitoba Soils as a Function of Soil Moisture Content, Temperature and Nutrient Levels. The objective of study three was to use the oriental mustard root bioassay to quantify the effects of soil properties, soil moisture, soil temperature and ammonium nitrate application on the bioavailability of imazamox:imazethapyr (1:1) and flucarbazone-sodium over time in Manitoba soils.

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2. SOIL PROPERTIES AFFECT IMAZAMOX:IMAZETHAPYR (1:1) AND FLUCARBAZONE-SODIUM PHYTOTOXICITY

2.1 Abstract

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are ALS inhibitor (Group 2) herbicides containing active ingredients that have a strong potential to persist in soil. These herbicide residues may damage subsequent sensitive crops when they are bioavailable to the plant by root uptake. Since there are limited studies on the phytotoxicity of imazamox:imazethapyr (1:1) and flucarbazone-sodium in Manitoba soils, this study applied an oriental mustard root bioassay to four Manitoba soil series ranging in texture from clay to sandy loam spiked with either imazamox:imazethapyr (1:1) or flucarbazone-sodium at 0, 3, 6, 12, 25, 50, 100 and 200 % of the commercial field rates. Root lengths of plants grown in soils were measured to calculate GR_{50} values (herbicide rates causing a 50 % growth reduction in root length). For two soils, the Lundar clay loam and the Stockton loamy sand, the root bioassay experiment was conducted under three ammonium nitrate treatments (i.e. application rates of 0, 75 and 150 kg N ha^{-1} to soil). In all soils, GR_{50} values were less for imazamox:imazethapyr (1:1) than for flucarbazone-sodium, demonstrating that oriental mustard is more sensitive to imazamox:imazethapyr (1:1) than flucarbazone-sodium residues in soil. Overall, no consistent significant trends were observed as to the effect of nitrogen on herbicide phytotoxicity because the effect was dependent on soil type, the concentration of nitrogen applied, and the concentration of herbicide applied. Both imazamox:imazethapyr (1:1)

and flucarbazone-sodium phytotoxicity significantly decreased with increasing soil organic carbon content, suggesting that herbicide sorption by soil decreased the bioavailability of herbicide residues to plants. This was further confirmed by quantifying the sorption of imazamox and imazethapyr by each of the four soils, and a negative correlation between sorption and phytotoxicity was observed. Although Group 2 herbicides have proven effective for weed control, these results demonstrate that in order to minimize the potential for crop damage following their use, well-planned rotations must be devised, particularly in coarser-textured soils with lower organic carbon contents.

2.2 Introduction

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are ALS inhibitor (Group 2) herbicides frequently used in western Canadian agriculture. The active ingredients imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) and imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5ethyl-3-pyridinecarboxylic acid) are imidazolinones most commonly used for the post emergence control of both grassy and broadleaf weeds in field peas, soybeans, fenugreek, alfalfa and imidazolinone-tolerant (Clearfield) canola and lentil (Anonymous, 2009a; Vencill, 2002a; Vencill, 2002b). In 2002, 24 % of producers surveyed in Manitoba used herbicide products containing these combined active ingredients (Leeson et al., 2002). Flucarbazone-sodium (1H-1,2,4-triazole-carboxamide,4,5-dihydro-3-methoxy-4-methyl5-oxo-N-[[2-(trifluoro-methoxy) phenyl]sulfonyl]-sodium salt), a sulfonylaminocarbonyltriazolinone, is a relatively new

post emergence herbicide used to control grassy and some broadleaf weeds in spring wheat and durum (Anonymous, 2009b; Vencill, 2002c). In western Canada, it is most frequently used in Manitoba where, in 2002, 29 % of producers surveyed used herbicide products containing this active ingredient (Leeson et al., 2002).

Group 2 herbicides, including active ingredients imazethapyr, imazamox and flucarbazone-sodium, have a strong potential to persist in soil past the season of application, potentially damaging subsequent sensitive crops (Jourdan et al., 1998b; Loux et al., 1989; Moyer and Esau, 1996; O'Sullivan et al., 1998). Herbicide residues in soil can be phytotoxic when they are bioavailable to the plant by root uptake, and this herbicide bioavailability is influenced by soil chemical and physical properties (Eliason et al., 2004; Ortega et al., 2004; Williams et al., 2002).

Bioassays are sensitive, simple techniques that can measure bioavailable herbicide residues in soil and aid in understanding the relation between soil properties and herbicide phytotoxicity. Eliason et al. (2004) tested five crops and determined that oriental mustard (*Brassica juncea*) root length was the best indicator for quantifying the bioavailable concentrations of flucarbazone-sodium residues in soil. They also found that flucarbazone-sodium phytotoxicity was much greater in five Saskatchewan soils ranging in texture from sandy loam to clay with organic carbon contents of 1.1 to 3.8 %, than in a Manitoba heavy clay soil with 4.3 % organic carbon content (Eliason et al. (2004). There have been no published studies on imazamox:imazethapyr (1:1) phytotoxicity in Manitoba soils.

According to the Manitoba Agriculture Yearbook, fertilizer has consistently been one of the largest farm operating expenditures in Manitoba, with 943,200 tonnes sold in

2004 (Anonymous, 2004). Of the fertilizer inputs applied, nitrogen (in various formulations) makes up the largest segment of fertilizer sales, at over 55 % in 2004 (Anonymous, 2004). Nitrogen levels have been shown to influence the susceptibility of plants to herbicides, however results differ based on plant species and herbicides applied (Cathcart et al., 2004; Chao et al., 1994; Lutman et al., 1975). No work has been conducted on the influence of soil nitrogen applications on the phytotoxicity of flucarbazone-sodium or imazamox:imazethapyr (1:1) herbicides.

The objective of this study was to use the oriental mustard root bioassay (Eliason et al. (2004) to quantify the effects of soil properties and ammonium nitrate application on the phytotoxicity of imazamox:imazethapyr (1:1) and flucarbazone-sodium in Manitoba soils.

2.3 Methods

2.3.1 Soil Series and Properties

Four surface soils (0-10 cm) were collected in southern Manitoba in the spring of 2004 from agricultural fields with no previous use of imazamox:imazethapyr (1:1) and flucarbazone-sodium. The four soils are a Red River clay (Red River C) from near Lowe Farm (SE 19-5-1W), a Lundar clay loam (Lundar CL) from near Warren (SE 12-14-2W), a Manitou silt loam (Manitou SL) from near La Riviere (SW 24-3-10W), and a Stockton loamy sand (Stockton LS) from near Neepawa (NW 13-14-14W) (Figure 2.1).

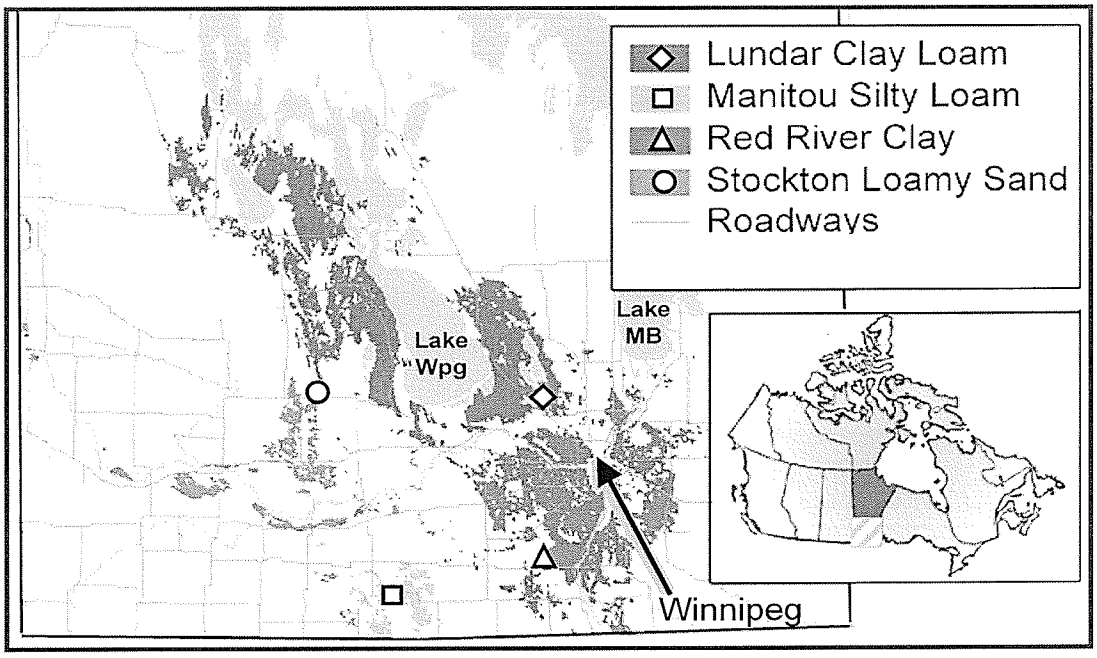

Figure 2.1 Map of southern Manitoba identifying the geographical location of the four sampling points and the area of each soil series.

Soils were air-dried and sieved (< 2 mm). Soil organic carbon content (SOC) was determined by dry combustion of 0.12 g of oven-dried soil with a LECO model CHN 600 Carbon-Hydrogen-Nitrogen Determinator (Nelson and Sommers, 1982). Inorganic carbon was first removed by adding 10 ml of 6 N HCl in distilled water to the soil, heating the slurry on a hot plate for 10 minutes and rinsing with 240 ml of distilled water (Tiessen et al., 1983). Soil texture was measured using the hydrometer method (Gee and Bauder, 1986) and an ASTM #152 H hydrometer (gL^{-1}). Soil pH was quantified using 5 g soil shaken for 30 minutes with 10 mL of 0.01 M $CaCl^2$ (Hendershot and Lalande, 1993). For the nutrient content analysis (AgVise Laboratories[1]), NO_3-N and SO_4-S were extracted with 0.001 M $CaCl_2$ and determined by the automated Cadmium Reduction Method 4500-NO_3 and the Turbidimetric Method 4500-SO_4^{2-} respectively (Clesceri et al.,

[1] AgVise Laboratories, 604 Highway 15 W, P.O. Box 510, Northwood, North Dakota USA, 58267.

1998). NH_4^+ was extracted with 2 N KCl and analyzed colorimetrically (Clesceri et al., 1998). P and K were extracted using the modified Kelowna method and determined by Stanneous Chloride Method 4500-P and the Flame Photometric Method 3500-K, respectively (Clesceri et al., 1998).

2.3.2 Herbicide bioassay studies without ammonium nitrate application

The oriental mustard root bioassay, adapted from Eliason et al. (2004), was used to quantify herbicide phytotoxicity in soils at field capacity. Silica sand[2] (#4095, 20-40 grains in^{-2}), an inert material, was used as a control substrate in the experiments, as herbicides are not sorbed by this sand. Field capacity of the four soils and silica sand was measured by determining the weight of water required to completely wet a sample of air-dried soil to the bottom of a 15 dram plastic vial without leaving standing water in the bottom of the vial after a 24-hour period (Eliason et al., 2004).

Stock solutions were prepared by diluting commercial formulations of imazamox:imazethapyr (1:1) (Odyssey[3]) or flucarbazone-sodium (Everest[4]) with deionized water to obtain concentrations of 0.15, 0.30, 0.60, 1.25, 2.50, 5.00, and 10.00 mg formulated product (f.p.) L^{-1}. Deionized water without herbicides was used as a control (0 mg f.p. L^{-1}). In order to account for differences in soil bulk densities across the five soils, weights of air-dried soil equivalent to 89 cm^3 were used in the bioassays (87 g Lundar CL, 75 g Manitou SL, 85 g Red River C, 108 g Stockton LS, 144 g silica sand).

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[2] Silica Sand (4095-01226), UNIMIN Corporation, Brock-White Construction Materials, 450 Sheppard Street, Winnipeg, Manitoba Canada, R2X 2P8.

[3] Odyssey, PCP#25111, 35% + 35% DF formulation, BASF Canada, 100 Milverton Drive, 5th Floor, Mississauga, Ontario Canada, L5R 4H1.

[4] Everest, PCP#26448, 75% DG formulation, Arysta LifeScience North America, 100 First Street, Suite 1700, San Francisco, California USA, 94105.

Soil was measured into 207 mL clear plastic Dixie® cups[5]. For the applications of either imazamox:imazethapyr (1:1) or flucarbazone-sodium to soil, aliquots (0.75 mL) of each stock solution were added to enough distilled water to bring soil to 100 % of its field capacity. For control treatments, only distilled water was used to bring the soil to the desired moisture level. Solutions were mixed in soil, yielding the equivalent application dosages of 0, 1.3, 2.5, 5.0, 10.5, 21.0, 42.0 and 84.0 mg f.p. m^{-3}. The application rate of 42.0 mg f.p. m^{-3} is approximately equivalent to the field application dosage of 30 g a.i. ha^{-1} for each herbicide (imazamox:imazethapyr (1:1) or flucarbazone-sodium), assuming the chemical is evenly distributed through the top 10 cm of soil. As such, these concentrations are here expressed as 0, 3, 6, 12, 25, 50, 100 and 200 % of the commercial field application dosage. Each treatment was replicated six times, and the entire experiment was duplicated, with the exception that: (1) the silica sand treatments were only included in the first experiment, as preliminary results indicated no significant difference between the silica sand and Stockton LS; and (2) the 200 % dosage was only included in the second experiment. After herbicide application, Dixie® cups of each dosage were placed into plastic trays, covered with the tray's lids, and left overnight in the dark to equilibrate.

Approximately 200 (0.6 g) oriental mustard seeds (*Brassica juncea*) variety AC Vulcan were placed into a Petri dish lined with filter paper wetted with 3 mL distilled water. Covered Petri dishes were left for 24-hours in the dark, and the filter paper was kept moist with distilled during germination. Seven pre-germinated seeds with radicles 1-3 mm long were planted into each cup of untreated or treated soil to a depth of 5 to 10

[5] CC7 Dixie® cups, Georgia-Pacific, Canada Wrap Limited, 196 Sutherland Avenue, Winnipeg, Manitoba Canada, R2W 5K7.

mm. Soil surfaces were covered with 15 g polyethylene plastic pellets[6] (i.e. one layer of pellets) to minimize moisture loss during growth. After planting, cups of each dosage were again placed into the plastic trays and covered with the tray's lids overnight.

Cups of oriental mustard seedlings were then randomly arranged in plastic trays and grown for five days at room temperature under fluorescent lights. Cups were randomized daily and the soils maintained at 100 % field capacity by adding distilled water (by weight) daily. After five days, whole seedlings were carefully removed from the soil and root lengths were measured to the nearest millimeter using a ruler. For each cup/replicate, root lengths were averaged over the seven plants, and percent of control was calculated for each replicate by:

$$Lt / L_0 \times 100\% \qquad [2.1]$$

where L_t is the root length measured in the imazamox:imazethapyr (1:1) or flucarbazone-sodium treated soil, and L_0 is the average root length measured in the untreated soil.

In order to establish dose responses for each soil and herbicide combination, data were subjected to nonlinear regression analysis using a 4 parameter log-logistic model (Seefeldt et al., 1995) in SAS version 9.1[7]:

$$y = C + [(D - C) / [1 + \exp[b(\log(x) - \log(I_{50}))]] \qquad [2.2]$$

where y = oriental mustard root length (expressed as percent of untreated control), x = herbicide dosage (expressed as percent of recommended field application dosage; a small positive value of 1.0 was assigned to 0 % dosage to calculate natural logarithms), C = lower limit (asymptote) of the response curve, D = upper asymptote of the response

[6] Polyethylene pellets, Westland Plastics Limited, 12 Rothwell Road, Winnipeg, Manitoba Canada, R3P 2H7.

[7] SAS version 9.1, 2000, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

curve, I_{50} = x-axis value that corresponds to the inflection point (i.e. "drop line" to x-axis) and b = slope of the curve at the I_{50} value. For each herbicide, individual curves for each soil type were statistically tested systematically for common C, common D, common b, and common I_{50}, using the lack-of-fit F test at the 0.05 level of significance as outlined by Seefeldt et al. (1995).

The I_{50} value corresponds to the inflection point of the curve, but because in most instances the curves' upper and lower limits are not 100 and 0, respectively, fitted I_{50} values do not necessarily represent the dosage of herbicide required to reduce root length by 50 % relative to the untreated control. Thus, GR_{50} values were calculated for each herbicide/soil combination by solving the above equation for x at y = 50 %:

$$x = I_{50} [((D - C) / (y - C) - 1) (1/b)] \qquad [2.3]$$

where $x = GR_{50}$, which is the herbicide dosage at y = 50 % of the untreated root length. These GR_{50} values were then correlated to soil clay content, organic carbon content, and soil pH by determining Pearson correlation coefficients in SAS version 9.1 at the 0.10 level of significance. The 0.10 level of significance was chosen, rather than the more traditional 0.05 level, because of the low number of data points (i.e. there were only four soils studied and thus only four data points could be used in the correlation analysis).

2.3.3 Herbicide bioassay studies with ammonium nitrate application

The oriental mustard root bioassay with ammonium nitrate application was completed for Lundar CL and Stockton LS treated with two dosages of ammonium nitrate (NH_4NO_3). Lundar CL and Stockton LS were chosen because of contrasting clay and organic carbon contents, but their similar pH and nutrient contents. Ammonium nitrate was used as a source of nitrogen as it has a lesser impact on soil pH than the more

commonly applied urea nitrogen (Hall and Curran, 2006). Since soil pH can affect herbicide sorption and bioavailability (Ahmad et al., 2001; Bresnahan et al., 2000), it was important to minimize any changes in soil pH with the addition of nitrogen.

An ammonium nitrate stock solution with a concentration of 25.5 g NH_4NO_3 L^{-1} (or 8.9 g N L^{-1}) was prepared in deionized water. Weights of air-dried soil equivalent to 89 cm^3 (87 g Lundar CL and 108 g Stockton LS) were measured into 207 mL clear plastic Dixie® cups. Aliquots (1.0 and 1.5 mL, respectively) of the ammonium nitrate stock solution were added to enough distilled water required to bring the soils to 75 % of their field capacities and then mixed with soil to establish concentrations of 214.3 and 428.6 g NH_4NO_3 m^{-3}. These concentrations are equivalent to typical low and high field application rates of 75 and 150 kg N ha^{-1}, assuming the nitrogen is evenly distributed through the top 10 cm of soil. This range accounts for approximately 95 % of field applications of nitrogen in Manitoba (D. Flaten, 2005, personal communications). Each cup of soil was covered with a Dixie® domed lid[8] with a 5 mm hole drilled in the centre to allow for gas diffusion. Soils were then incubated at 25°C for two weeks to allow for the processes of nitrification and denitrification to occur before herbicide application. During incubation, cups of soil were watered (by weight) to 75 % field capacity when 10 % moisture loss occurred (every 3 days for Stockton LS and 4 days for Lundar CL). Soil pH was quantified 24 hours after the addition of ammonium nitrate and following the two week incubation period using 5 g soil shaken for 30 minutes with 10 mL of 0.01 M $CaCl^2$ (Hendershot and Lalande, 1993). Following incubation of two weeks, aliquots (0.75 mL) of herbicide solutions were added to enough distilled water to bring soils to 100 % of

[8] DF57 Dixie® domed lid, Georgia-Pacific, Canada Wrap Limited, 196 Sutherland Avenue, Winnipeg, Manitoba Canada, R2W 5K7.

their field capacities. The bioassay experiment and statistical analysis were then completed as described above (2.3.2). Each treatment was replicated six times and the experiment was only completed once.

2.3.4 Herbicide Sorption to Soil

In order to facilitate the interpretation of the bioassay experiments, the batch equilibrium technique was used to determine the Freundlich distribution coefficient (Kf), as this is a good measure of the extent of herbicide sorption by soil (Wauchope et al., 2002). For these analyses, herbicide sorption by soil was determined for imazamox and imazethapyr[9] separately. Herbicide solutions were prepared in 0.01M CaCl at herbicide concentrations of 4.4, 8.8, 17.5, 35.0 and 70.0 $\mu g\ L^{-1}$. Based on the herbicide solutions applied in bioassay experiments described above, the 8.8 $\mu g\ L^{-1}$ solution applied to 5 g soil in a 10 mL aliquot, is approximately equivalent to the concentration of imazamox or imazethapyr found in the recommended field application dosages of imazamox:imazethapyr (1:1) (the herbicide treatment referred to as 100 %). The imazamox solutions contained 505, 1015, 2025, 4005 and 8040 DPM radioactivity, respectively, and the imazethapyr solutions contained 1860, 3710, 7660, 15 330 and 30 680 DPM radioactivity, respectively. For each concentration, 5 g air-dried soil was combined with the herbicide solution (10 mL) in Teflon tubes in duplicates and rotated for 24 hours in the dark to establish equilibrium. The soil slurry was then centrifuged for 10 minutes at 10,000 rev min^{-1} after which 1 mL sub-samples of supernatant (duplicates)

[9] ^{14}C-imazamox BAS 720 H, specific activity 0.049 mCi mg^{-1}, 99.6% radiopurity and ^{14}C-imazethapyr BAS 685 H, specific activity 0.202 mCi mg^{-1}, 97.1% radiopurity, BASF Corporation, 26 Davis Drive, Research Triangle Park, North Carolina USA, 27709.

were removed from each tube. Scintillation cocktail[10] (10 mL) was added to the sub-samples to determine the amount of herbicide in equilibrium solution by Liquid Scintillation Counting (LSC) with automated quench correction (#H method) (LS 7500 Beckman Instruments, Fullerton, CA) and a maximum counting time of 10 minutes. The Kf ($\mu g^{1-1/n}\ g^{-1}\ mL^{1/n}$) was quantified by nonlinear regression using the empirical Freundlich equation in the log transformation form in Sigma Plot version 6.00[11] with the *Cs* (the amount of herbicide sorbed to the soil) and *Ce* (the concentration of herbicide in the equilibrium solution) averaged over replicates and yielding one Kf value for each soil and herbicide combination:

$$\log Cs = \log Kf + \frac{1}{n}\log Ce \qquad [2.4]$$

where *1/n* is the dimensionless Freundlich constant describing nonlinearity. The units of *Cs* ($\mu g\ kg^{-1}$) and *Ce* ($\mu g\ L^{-1}$) ensure that the lines of all isotherms cross Ce = 1, an important consideration in calculating Kf (Bowman 1981, Bowman 1982). In the Stockton LS/imazamox experiments, some of the Cs values in the 200, 400 and 800 % application rates were negative. These were set to zero, indicating no sorption of imazamox to the Stockton LS. Kf values, calculated for each soil/herbicide were then correlated to soil properties (clay content, organic carbon content and pH) and imazamox:imazethapyr (1:1) GR_{50} values by determining Pearson correlation coefficients (SAS version 9.1) at the 0.10 level of significance.

The commercial formulation of imazamox:imazethapyr (1:1) is composed of 35 % imazethapyr and 35 % imazamox. As such, the two active ingredients are applied

[10] 30 % Scintisafe scintillation cocktail, Fisher Scientific, Fairlawn, NJ

[11] Sigma Plot version 6.00, 1986-2000, SPSS Inc., 233 S. Wacker Drive, 11th floor Chicago, Illinois 60606

together in the field, which could result in possible interactions in how the chemicals react with soil. Thus, the effect of imazethapyr in solution on imazamox sorption by soil, and of imazamox in solution on imazethapyr sorption by soil was also examined. This was done by preparing two separate stock solutions each consisting of commercial formulations of imazamox:imazethapyr (1:1) at 37.5 μg f.p. L^{-1} (150 % of its field application dosage) and either 4.4 μg L^{-1} [U-^{14}C] imazamox (50 % of its field application dosage) or 4.4 μg L^{-1} [U-^{14}C] imazethapyr (50 % of its field application dosage). The mixed stock solutions were at concentrations of 8.8 μg L^{-1} imazamox and 6.6 μg L^{-1} imazethapyr, and at concentrations of 6.6 μg L^{-1} imazamox and 8.8 μg L^{-1} imazethapyr, respectively. Using these solutions, sorption was determined using the batch equilibrium technique, described above, and reported as the sorption distribution coefficient (Kd). Kd (L kg^{-1}) was calculated by:

$$Kd = Cs / Ce \qquad [2.5]$$

To determine whether there was an effect of imazethapyr in solution on imazamox sorption by soil, ANOVA (PROC GLM) was run on Kd values determined with the mixed stock solution (8.8 μg L^{-1} imazamox, including 4.4 μg L^{-1} [U-^{14}C] imazamox, and 6.6 μg L^{-1} imazethapyr) versus the stock solution containing only 4.4 μg L^{-1} [U-^{14}C] imazamox (50 % of its field application dosage). To determine whether there was an effect of imazamox in solution on imazethapyr sorption by soil, ANOVA (PROC GLM) was run on Kd values determined with the mixed stock solution (6.6 μg L^{-1} imazamox and 8.8 μg L^{-1} imazethapyr, including 4.4 μg L^{-1} [U-^{14}C] imazethapyr) versus the stock solution containing only 4.4 μg L^{-1} [U-^{14}C] imazethapyr (50 % of its field application dosage). Any differences observed are thus a result of both co-application of

the two active ingredients, as well as the greater application rates in the mixed stock solutions (100 % versus 50 % of the field application rates). However, in both mixed and individual stock solutions, only the radiolabelled portion of the solution of 4.4 $\mu g\ L^{-1}$ [U-^{14}C] is measured through the batch equilibrium technique.

In order to ensure that there was no sorption of imazamox or imazethapyr to the plastics used in the bioassay experiments (Dixie cups, mixing containers, polyethylene pellets), preliminary batch equilibrium experiments were completed as described above in replicates of four. The 100 % application rate of either imazamox or imazethapyr was added to Teflon tubes containing each type of plastic alone or in combination with soil (to determine if there was an interaction between the soil and plastics). Sorption of either herbicide to plastics mixed with soil was no greater than that of soil alone, suggesting that the percent sorption of imazamox or imazethapyr to the various plastics is negligible.

2.4 Results and Discussion

2.4.1 Soil Properties Affect Phytotoxicity of Imazamox:Imazethapyr (1:1) and Flucarbazone-sodium in Soil

Soils had a wide range of properties (Table 2.1) but, in all cases, the response of oriental mustard root length to increasing dosages of imazamox:imazethapyr (1:1) or flucarbazone-sodium was described very well by the log-logistic model (Table 2.2, Figure 2.2). For response to imazamox:imazethapyr (1:1), all dose response curves had the same lower (*C*) and upper (*D*) limits. Four of the five soil treatments (Manitou SL, Red River C, Stockton LS and Silica S) had the same slope (*b*), as depicted by the parallel curves (Figure 2.2 A). The Stockton LS and Silica S I_{50} values were notably

lower than the other three soils, indicating that imazamox:imazethapyr (1:1) is more phytotoxic to oriental mustard in Stockton LS and Silica S as compared to the other soil treatments. Manitou SL and Red River C series had all parameters common, as did Stockton LS and Silica S, thus only one curve was drawn for each pair (Figure 2.2 A). For response to flucarbazone-sodium, all dose response curves had the same lower (*C*) and upper (*D*) limits. Lundar CL, Stockton LS and Silica S series shared the same slope, as did the remaining two soil treatments (Manitou SL and Red River C) (Figure 2.2 B). I_{50} values were significantly different for all soils except Stockton LS and Silica S, which had all parameters common. Overall, flucarbazone-sodium was less phytotoxic to oriental mustard than imazamox:imazethapyr (1:1) by at least a factor of three (Table 2.2), and in all soils, imazamox:imazethapyr (1:1) phytotoxicity was observed at even the lowest rate applied (Figure 2.2 A).

Table 2.1 Selected soil properties for the four Manitoba soil series studied.

Soil Property	Lundar Clay Loam	Manitou Silt Loam	Red River Clay	Stockton Loamy Sand
Clay Content (%)	30.6	25.7	53.1	9.7
Organic Carbon Content (%)	3.65	4.54	3.93	0.52
pH (in CaCl2)	7.3	5.8	7.4	7.2
Field Capacity (%)	55.0	60.6	55.9	32.0
Bulk Density (g cm-3)	0.97	0.84	0.95	1.21
Nitrogen (kg ha^{-1})	27.5	75.0	127.5	19.0
Phosphorus (ppm)	18.0	29.5	24.0	8.5
Potassium (ppm)	497.0	744.5	784.5	95.0
Sulphur (kg ha^{-1})	6.0	15.0	10.0	6.0

Table 2.2 Equations describing the response of oriental mustard root length grown in four Manitoba soils and a control soil (Silica Sand) treated with increasing dosages of imazamox:imazethapyr (1:1) or flucarbazone-sodium. Parameter estimates (C, D and b) are for root lengths expressed as a percentage of untreated controls ± standard errors. Refer to Materials and Methods for a description of the log-logistic model fitted.

Herbicide	Soil Series	C[a] ± SE	D ± SE	b ± SE	I_{50}[b] ± SE	GR_{50}[c]
Imazamox: imazethapyr (1:1)	Lundar CL	16.7 ± 1.2	104.7 ± 1.9	1.0 ± 0.1	10.1 ± 1.0	16.6
	Manitou SL	16.7 ± 1.2	104.7 ± 1.9	1.7 ± 0.1	15.1 ± 0.8	20.2
	Red River C	same as Manitou Silt Loam				
	Stockton LS	16.7 ± 1.2	104.7 ± 1.9	1.7 ± 0.1	4.1 ± 0.2	5.5
	Silica S	same as Stockton Loamy Sand				
Flucarbazone-sodium	Lundar CL	14.5 ± 1.4	98.1 ± 0.8	2.0 ± 0.1	31.3 ± 1.8	36.4
	Manitou SL	14.5 ± 1.4	98.1 ± 0.8	3.5 ± 0.3	60.7 ± 2.6	66.3
	Red River C	14.5 ± 1.4	98.1 ± 0.8	3.5 ± 0.3	42.7 ± 1.8	46.6
	Stockton LS	14.5 ± 1.4	98.1 ± 0.8	2.0 ± 0.1	16.0 ± 0.7	18.6
	Silica S	same as Stockton Loamy Sand				

[a] Statistical differences between parameter estimates were determined using the lack-of-fit F test at the 0.05 level of significance (refer to Materials and Methods). R^2 values for both herbicides were 0.99.
[b] I_{50} values are a percentage of the recommended field application dosage.
[c] GR_{50} values were calculated by solving the log-logistic model for x at y = 50 % (refer to Materials and Methods).

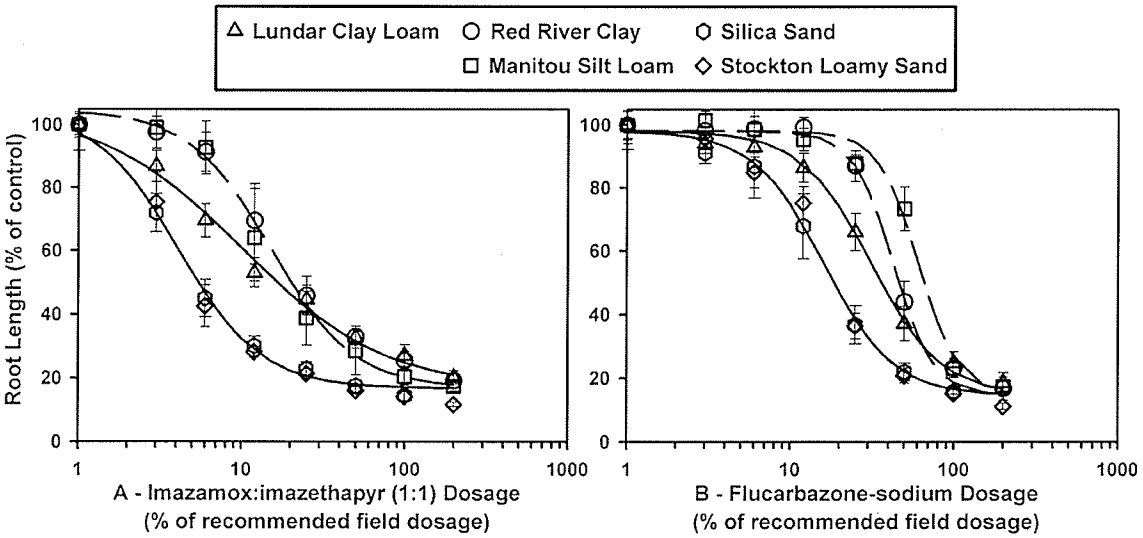

Figure 2.2 Dose response curves of oriental mustard root lengths (percentage of untreated control) grown in four Manitoba soils and a control soil (Silica Sand) containing either A) imazamox:imazethapyr (1:1) or B) flucarbazone-sodium herbicide. Symbols are the means ± standard deviations. Refer to Table 2.2 for parameter estimates of the log-logistic model fitted. Curves were significantly different according to the lack-of-fit F test (refer to Materials and Methods).

Both imazamox:imazethapyr (1:1) and flucarbazone-sodium showed good correlations between GR_{50} and organic carbon content (Table 2.3), however the correlations were only significant at the 0.01 and 0.11 levels, respectively. No significant correlation was observed between GR_{50} and clay content or pH (Table 2.3). These findings are in agreement with Eliason et al. (2004) who observed a strong significant correlation ($p < 0.01$) between I_{50} values for flucarbazone-sodium and organic carbon content, but no significant correlation with clay content ($p=0.90$) or pH ($p = 0.39$). As flucarbazone-sodium is a recently commercialized herbicide, no other studies examining the correlation of flucarbazone-sodium phytotoxicity and soil properties have been published to date.

Table 2.3 Correlation analysis between imazamox:imazethapyr (1:1) or flucarbazone-sodium calculated (using Equation 2.4) GR_{50} values and soil properties. Correlation coefficients are followed by probabilities in parentheses.

Soil Property	Imazamox:imazethapyr (1:1) GR_{50}	Flucarbazone-sodium GR_{50}
Clay Content	r = 0.78 (0.22)	r = 0.44 (0.56)
Organic Carbon Content	r = 0.99 (0.01)	r = 0.89 (0.11)
Soil pH	r = -0.35 (0.65)	r = -0.76 (0.25)

2.4.2 Nitrogen Application and Phytotoxicity

The application of ammonium nitrate to the Lundar CL demonstrated a slight decrease in pH at 24 hours as compared to the Lundar CL not receiving the amendment, but the fertilizer had a lesser impact on the pH of the Stockton LS (Table 2.4). It is known that ammonium-based nitrogen fertilizers can lower soil pH (Hall and Curran, 2006). However, after two weeks incubation, the pH of the soils receiving fertilizer was equal to or approached that of the control soil (no fertilizer) (Table 2.4).

Table 2.4 pH of soils treated with or without ammonium nitrate nitrogen, incubated overnight and for two weeks.

Incubation Period	Lundar Clay Loam (kg N ha^{-1})			Stockton Loamy Sand (kg N ha^{-1})		
	0	75	150	0	75	150
24 hours	7.1	7.0	6.8	7.1	7.1	7.2
2 weeks	7.2	7.1	7.1	7.2	7.2	7.2

The response of oriental mustard root length to increasing dosages of imazamox:imazethapyr (1:1) or flucarbazone-sodium grown in soil treated with or without ammonium nitrate was described very well by the log-logistic model (Table 2.5, Figure 2.3). For response to imazamox:imazethapyr (1:1) in Lundar CL, all nitrogen dose response curves had the same lower (*C*) and upper (*D*) limits (Table 2.5). Both the 75 and 150 kg N ha^{-1} nitrogen-treated curves had all parameters common, thus only one curve was drawn for these two nitrogen treatments (Figure 2.3 A). Oriental mustard plants grown in nitrogen-treated Lundar CL showed greater sensitivity to imazamox:imazethapyr (1:1) than when grown in Lundar CL containing no added nitrogen (i.e. the I_{50} values of the 75 and 150 kg N ha^{-1} treatments were lower than that of the 0 kg N ha^{-1} treatment). A similar trend was observed by Nalewaja et al. (1990) who observed increasing kochia control by imazethapyr (i.e. increased sensitivity to imazethapyr) in a loamy sand soil treated with increasing rates of ammonium nitrate. They attributed this to plant stress when grown in soils with low nitrogen. In contrast, plants grown in Stockton LS treated with nitrogen showed lesser sensitivity to imazamox:imazethapyr (1:1) as compared to plants grown in Stockton LS containing no added nitrogen, although there was a smaller difference between the two nitrogen-treated curves than was observed for Lundar CL (Figure 2.3 A).

Table 2.5 Equations describing the response of oriental mustard root length to increasing dosages of imazamox:imazethapyr (1:1) or flucarbazone-sodium grown in two Manitoba soils treated with or without ammonium nitrate nitrogen. Parameter estimates are for root lengths expressed as a percentage of untreated controls ± standard errors. Refer to Materials and Methods for a description of the log-logistic model fitted.

Herbicide	Soil Series & Nitrogen Treatment (kg N ha^{-1})		C[a] ± SE	D ± SE	b ± SE	I_{50}[b] ± SE
Imazamox: imazethapyr (1:1)	Lundar CL	0	14.9 ± 1.6	110.5 ± 4.3	0.9 ± 0.1	9.0 ± 1.3
		75	14.9 ± 1.6	110.5 ± 4.3	1.5 ± 0.2	5.0 ± 0.4
		150	same as 75 kg N ha^{-1}			
	Stockton LS	0	13.1 ± 1.1	104.3 ± 2.0	2.0 ± 0.2	4.4 ± 0.3
		75	13.1 ± 1.1	104.3 ± 2.0	2.0 ± 0.2	5.6 ± 0.3
		150	same as 75 kg N ha^{-1}			
Flucarbazone-sodium	Lundar CL	0	12.6 ± 1.8	98.1 ± 1.1	1.9 ± 0.1	31.9 ± 1.4
		75	same as 0 kg N ha^{-1}			
		150	12.6 ± 1.8	98.1 ± 1.1	1.9 ± 0.1	27.3 ± 1.5
	Stockton LS	0	11.7 ± 1.1	97.5 ± 1.1	2.4 ± 0.2	18.5 ± 0.7
		75	same as 0 kg N ha^{-1}			
		150	11.7 ± 1.1	97.5 ± 1.1	3.7 ± 0.5	21.4 ± 0.8

[a] Statistical differences between parameter estimates were determined using the lack-of-fit F test at the 0.05 level of significance (refer to Materials and Methods). R^2 values for all herbicide/soil treatments were 0.99.
[b] I_{50} values are a percentage of the recommended field application dosage.

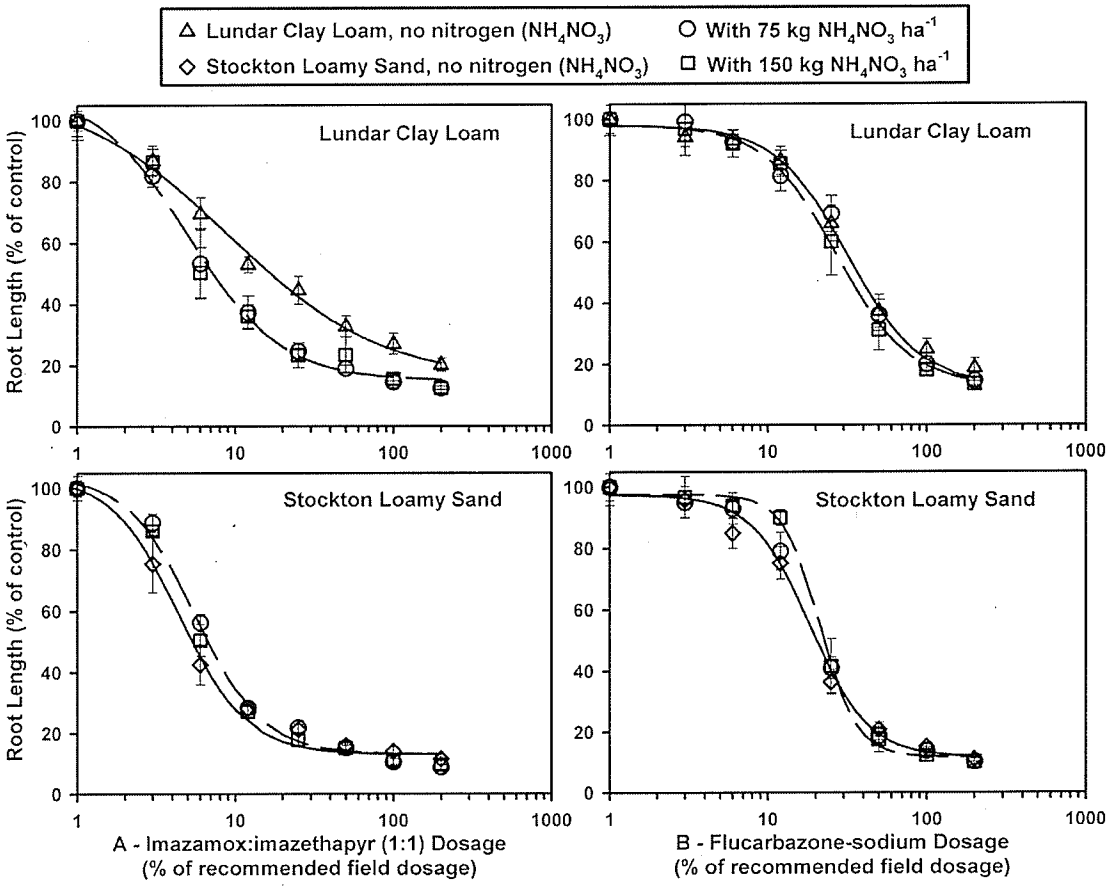

Figure 2.3 Dose response curves of oriental mustard root lengths (percentage of untreated control) to either A) imazamox:imazethapyr (1:1) or B) flucarbazone-sodium herbicide grown in two Manitoba soils treated with or without ammonium nitrate nitrogen. Symbols are the means ± standard deviations. Refer to Table 2.5 for parameter estimates of the log-logistic model fitted. Curves were significantly different according to the lack-of-fit *F* test (refer to Materials and Methods).

For response to flucarbazone-sodium in both Lundar CL and Stockton LS, the 0 and 75 kg N ha^{-1} treatments shared all parameters (Table 2.5). Thus only higher dosages of ammonium nitrate affected oriental mustard sensitivity to flucarbazone. As observed with imazamox:imazethapyr (1:1), I_{50} values indicate that the addition of high dosages of nitrogen to Lundar CL increased the phytotoxicity of flucarbazone, while the addition of nitrogen to Stockton LS reduced the phytotoxicity (Table 2.5). In Stockton LS, the addition of high rates of nitrogen (150 kg N ha^{-1}) reduced the phytotoxicity when rates of flucarbazone-sodium in soil were less than 30 % of the field application dosage, while at higher dosages of flucarbazone-sodium (>30 %), phytotoxicity of flucarbazone-sodium to oriental mustard was increased in the 150 kg N ha^{-1} treated soils (i.e. the difference in slopes between the 0-75 kg ha^{-1} and 150 kg ha^{-1} caused the curves to cross) (Figure 2.3 B). Overall, no consistent significant trends were observed as to the effect of nitrogen on the phytotoxicity of imazamox:imazethapyr (1:1) or flucarbazone-sodium to oriental mustard. More soils and nitrogen treatments would be required to find more definitive results.

2.4.3 Sorption and Phytotoxicity

Based on the experiments examining the effect of herbicide co-application on sorption, the addition of imazethapyr to solutions containing imazamox had no significant effect on sorption of imazamox to soil ($p = 0.15$), while the addition of imazamox did significantly increase imazethapyr sorption to soil ($p = 0.02$). Averaged over the four soils, Kd of imazethapyr applied alone was 0.79 ± 0.71 L kg^{-1}, compared to 0.84 ± 0.70 L kg^{-1} when imazamox (Odyssey) was added. The actual differences were very small and

the standard deviation among soils were large, hence it can be concluded that there was very little interaction of the two active ingredients on sorption.

When either herbicides were applied alone, Kf values ranged from 0.22 to 0.96 $\mu g^{1-1/n}\ g^{-1}\ mL^{1/n}$ for imazamox and from 0.23 to 1.89 $\mu g^{1-1/n}\ g^{-1}\ mL^{1/n}$ for imazethapyr (Table 2.6). This is within the range of Kf values observed for imazamox by Bresnahan et al. (2002) in Minnesota soils (0.26-1.30 $mg^{1-1/n}\ kg^{-1}\ L^{1/n}$) and of Kd values observed for imazethapyr by Ahmad et al. (2001) in soils from Pakistan and Australia (0.02-6.94 L kg^{-1}). Although flucarbazone-sodium sorption was not evaluated, Koskinen et al. (2002) reported flucarbazone-sodium Kd values of 0.65 mL g^{-1} in a clay loam soil and 0.11 mL g^{-1} in a loamy sand after no incubation. Thus, it appears that flucarbazone-sodium sorption would be numerically similar to that of imazamox:imazethapyr (1:1). In all soils, imazethapyr sorption was greater than imazamox sorption (Table 2.6). For imazamox, the slopes of the sorption isotherms (1/n) were less than unity, ranging from 0.48 to 0.90. These values ($1/n < 1$) demonstrate that the saturation of the sorption sites limited further sorption as herbicide concentration increased. In other words, the Freundlich fitting of the imazamox isotherm was L-type (Giles et al., 1960). For imazethapyr, 1/n values were linear, as they were close to 1 (0.90 to 1.01). Thus the Freundlich fitting of the imazethapyr isotherm was C-type (Giles et al., 1960), indicating no effect of concentration on sorption. Linear sorption isotherms were previously observed by Ahmad et al. (2001) and Bresnahan (2000) for imazethapyr.

Table 2.6 Freundlich sorption coefficients (*Kf*) and slopes of the Freundlich isotherms (*1/n*) for imazamox or imazethapyr applied to four Manitoba soils. Refer to Materials and Methods for a description of the sorption distribution coefficient.

Active Ingredient	Soil Series	Kf ± SE	1/n	R2
Imazamox	Lundar CL	0.28 ± 0.07	0.82	0.97
	Manitou SL	0.96 ± 0.09	0.90	>0.99
	Red River C	0.36 ± 0.07	0.84	0.98
	Stockton LS	0.22 ± 0.07	0.48	0.86
Imazethapyr	Lundar CL	0.44 ± 0.04	1.01	>0.99
	Manitou SL	1.89 ± 0.10	1.00	>0.99
	Red River C	0.67 ± 0.06	0.95	>0.99
	Stockton LS	0.23 ± 0.07	0.90	0.96

There was a strong significant association between imazamox and imazethapyr sorption (Table 2.7), indicating that the two active ingredients of imazamox:imazethapyr (1:1) act very similarly across the four soils studied, even though there was more sorption of imazethapyr to soil than imazamox. The sorption of imazamox and imazethapyr was significantly negatively associated with soil pH, but not to other soil properties (Table 2.7). This is in agreement with other findings where the sorption of either imazamox or imazethapyr increased with decreasing soil pH (Ahmad et al., 2001; Bresnahan et al., 2002; Oliveira et al., 2001; Vencill, 2002ab). This is likely due to the amphoteric nature of these chemicals, such that, at low pH values, a relative greater portion of molecules are present as cations and therefore preferentially sorbed by negatively charged clay and organic matter surfaces. In contrast, at higher pH levels, more molecules are in the anionic form and repulsed by negatively charged clay and organic matter surfaces. Even though in this study, there were not significant influences of other soil properties on herbicide sorption, Ahmad et al. (2001) reported that imazethapyr sorption increases with increasing soil organic matter and clay contents.

Table 2.7 Correlation analysis between imazamox or imazethapyr Kf values and GR_{50} values and soil properties. Correlation coefficients are followed by probabilities in parentheses.

Soil Property	Imazamox Kf	Imazethapyr Kf
Imazamox Kf	r = 1.00	r > 0.99 (<0.01)
Imazethapyr Kf	r > 0.99 (<0.01)	r = 1.00
Clay Content	r = 0.02 (0.98)	r = 0.10 (0.90)
Organic Carbon Content	r = 0.63 (0.37)	r = 0.68 (0.32)
Soil pH	r = -0.97 (0.03)	r = -0.94 (0.06)
Imazamox:Imazethapyr (1:1) GR_{50}	r = 0.58 (0.42)	r = 0.64 (0.63)

For both imazamox and imazethapyr, the Manitou SL series (lowest pH, highest organic carbon) showed the greatest sorption, followed by the Red River C, Lundar CL and Stockton LS (Table 2.6). A similar trend was found with the phytotoxicity of imazamox:imazethapyr (1:1) to oriental mustard root length in the four soils (Table 2.2), where GR_{50} values decreased in the order of Manitou SL = Red River C > Lundar CL > Stockton LS. This appears to suggest that increasing sorption reduces phytotoxicity in soils. However, the associations between Kf values and GR_{50} were not significant (Table 2.7), perhaps because of the small number of soils used (4 soils).

In order to study the relation more directly, and with more data points, correlation analysis was also conducted between *Ce*, expressed as a percentage of the field application rate, and the observed root length of the oriental mustard plants grown in soil with the same application rate of imazaomox:imazethapyr (1:1) (from bioassay experiments). Since three of the application rates that were used in the bioassay and sorption experiments are the same (50, 100 and 200 % of the field application rate), this analysis allows for more data points to be analyzed for each soil/herbicide combination (4

soils x 3 application rates = 12 data points). *Ce* represents the bioavailable herbicide, as it is the amount of herbicide remaining in solution after sorption has reached equilibrium. When combined over all soils, a strong significant negative correlation was observed (Table 2.8). This indicates that as herbicide sorption to soil increases, the bioavailability of herbicide residues for plant uptake decreases (i.e. decreased *Ce*), resulting in lower phytotoxicity (i.e. increased root length).

Table 2.8 Correlation analysis between concentrations of imazamox or imazethapyr remaining in solution after sorption (expressed as a percent of the field application dosage) and the average length of oriental mustard roots treated with imazamox:imazethapyr (1:1) at the same dosages.

	Imazamox in Solution	Imazethapyr in Solution
Oriental Mustard Root Length (mm)	r = -0.62 (0.03)	r = -0.63 (0.03)

2.5 Conclusions

Imazamox:imazethapyr (1:1) and flucarbazone-sodium, Group 2 herbicides used frequently in western Canadian agriculture, have a high potential to persist in soil past the season of application. If these soil residues are bioavailable to subsequent sensitive crops, a significant reduction in yield or even crop loss may occur. Bioassays are important tools that can be used to better understand the effect or phytotoxicity of imazamox:imazethapyr (1:1) and flucarbazone-sodium to sensitive crops grown on different soil series. Results of this study indicate that phytotoxicity can be at least partially predicted by the sorptive capacity of a soil. When a herbicide is sorbed to the soil, it is removed from the bioavailable portion of the soil solution and thus

phytotoxicity is reduced. Although soil properties have differing effects on phytotoxicity and sorption, generally, increases in soil organic carbon content and clay content, and decreases in pH were observed to increase sorption and reduce phytotoxicity of imazamox:imazethapyr (1:1) and flucarbazone-sodium in Manitoba soils. The impact of the addition of ammonium nitrate to soil on imazamox:imazethapyr (1:1) and flucarbazone-sodium phytotoxicity must be further investigated as the results of this study indicated that there were significant increases or decreases in phytotoxicity depending on soil texture and the amounts of fertilizer or herbicides applied to soil.

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3. IMAZAMOX:IMAZETHAPYR (1:1) AND FLUCARBAZONE-SODIUM "STACKING" IN MANITOBA SOILS

3.1 Abstract

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are active ingredients in Group 2 herbicides products. These active ingredients are relatively persistent in soil and hence could damage subsequent sensitive crops when bioavailable to the plant. With the increased use of a variety of Group 2 herbicides in Prairie agriculture, concerns have been raised regarding the potential build-up of ALS inhibitor residues in soils because "stacking" could result in a greater crop injury compared to that induced by residuals of individual herbicides in soil. The objective of this study was to quantify interaction responses of imazamox:imazethapyr (1:1) and flucarbazone-sodium applied to four Manitoba soils using an oriental mustard root bioassay. Herbicides were either applied to soil alone at 0, 3, 6, 12, 25, 50, 100 and 200 % of their field application dosage, or in combination with one herbicide at 25 % of its field application dosage and the other herbicide at 0, 3, 6, 12, 25, 50, and 100 % of its field application dosage. The "*observed responses*" were determined by measuring plant root length responses when soil contained the combined herbicide residues. The "*expected responses*" were calculated based on (Colby, 1967) by considering plant root length in soils that contained either imazamox:imazethapyr or flucarbazone-sodium alone. Results demonstrated that depending on soil characteristics and the amounts of herbicide residues in soil, the effects were either additive (i.e. there was a good agreement between the "*observed*" and "*expected*" results), antagonistic (i.e. the crop injury was less than expected) or

synergistic (i.e. the crop injury was greater than expected). In a typical field situation whereby a producer applied flucarbazone-sodium to a soil containing imazamox: imazethapyr (1:1) residues from an application in the previous year, stacking would only occur in the Manitou silt loam series. In contrast, when a producer would apply imazamox:imazethapyr (1:1) to soils containing flucarbazone-sodium residues stacking would occur in a wider range of soils: Lundar clay loam, Manitou silt loam and Red River clay.

3.2 Introduction

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are ALS inhibitor (Group 2) herbicides frequently used in western Canadian agriculture. Imazamox:imazethapyr (1:1) are imidazolinones and applied post emergence to field peas, soybeans, fenugreek, alfalfa and imidazolinone-tolerant (Clearfield) canola and lentils to control both grassy and broadleaf weeds (Anonymous, 2009a; Vencill, 2002a; Vencill, 2002b). In 2002, its estimated use in Manitoba was approximately 24 % of fields (Leeson et al., 2002). Flucarbazone-sodium is a relatively new post emergence herbicide used to control grassy and some broadleaf weeds in spring wheat and durum (Anonymous, 2009b; Vencill, 2002c). The herbicide is a sulfonylaminocarbonyltriazolinone and more frequently used in Manitoba (29 % of respondents used it in 2002) than in the other two Prairie provinces (Leeson et al., 2002).

Imazamox:imazethapyr (1:1), flucarbazone-sodium, and certain other Group 2 herbicides have a high potential to persist in soil past the season of application, potentially damaging subsequent sensitive crops (Jourdan et al., 1998; Loux et al., 1989;

Moyer and Esau, 1996; O'Sullivan et al., 1998). These soil residues can be phytotoxic to successively planted crops when they are bioavailable to the plant via root uptake, and this bioavailability is influenced by soil chemical and physical properties. Bioassays are sensitive, simple techniques that can estimate bioavailable herbicide residues in soil and aid in understanding the relation between soil properties and herbicide phytotoxicity. Eliason et al. (2004) tested various crops to determine which could best provide a sensitive, accurate bioassay for the detection of flucarbazone-sodium in soil. Of the five crops they tested, oriental mustard (*Brassica juncea*) root length was found to be the best indicator. Eliason et al. (2004) measured flucarbazone-sodium phytotoxicity and persistence in five Saskatchewan soils and one Manitoba soil, and found that its half-life was significantly greater in the Manitoba soil. Because flucarbazone-sodium is more persistent in Manitoban soils, there is a greater chance that the flucarbazone-sodium residue will carry-over into years when other ALS inhibitor (Group 2) herbicides are applied to the same soil.

Johnson et al. (2005) reported increasing frequencies of "back-to-back" ALS inhibitor usage on the Prairies. In 2002, 37 % of Manitoba respondents to Leeson et al.'s (2002) Weed Survey Questionnaire applied soil residual ALS inhibitors in successive years. In 2001, the Weed Subcouncil of the Saskatchewan Advisory Council on Soils and Agronomy raised concerns about these repeated applications after field agronomists reported increased field pea (*Pisum sativum* L.) injury when fields were treated with imazamox:imazethapyr (1:1) following flucarbazone-sodium applications in the previous year (Johnson et al., 2005). The repeated use of residual herbicides resulting in either additive or synergistic phytotoxicity to rotational crops, has been termed herbicide

residue "*stacking*", as defined by Johnson et al. (2005). Johnson and other researchers are currently conducting field and laboratory studies with Saskatchewan and Alberta soils to investigate the potential risk associated with ALS inhibitor stacking. To date little work has been conducted using Manitoba soils.

The objective of this study was to quantify the interaction responses of imazamox:imazethapyr (1:1) and flucarbazone-sodium applied to four southern Manitoba soils using the oriental mustard root bioassay.

3.3 Methods

3.3.1 Soil Series and Properties

Four surface soils (0-10 cm), with varying properties and no history of imazamox:imazethapyr (1:1) or flucarbazone-sodium application were collected from across southern Manitoba as described in Chapter 2. Soils are here identified by their soil series classification and soil texture: Lundar Clay Loam (Lundar CL), Manitou Silt Loam (Manitou SL), Red River Clay (Red River C) and Stockton Loamy Sand (Stockton LS). Soils were air-dried and sieved (< 2 mm) prior to soil property (Table 2.1) and bioassay analyses.

As described in Chapter 2, soil organic carbon content (SOC) was determined by dry combustion (Nelson and Sommers, 1982); soil texture was measured using the hydrometer method (Gee and Bauder, 1986); soil pH was determined with $CaCl_2$ (Hendershot and Lalande, 1993); and NO_3-N, SO_4-S, P and K were quantified using the automated Cadmium Reduction Method 4500-NO_3, the Turbidimetric Method 4500-SO_4^{2-}, the Stanneous Chloride Method 4500-P and the Flame Photometric Method 3500-

K, respectively (Clesceri et al., 1998). Field capacity (as a percent) was measured by determining the weight of water required to completely wet a 35 g sample of air-dried soil to the bottom of a 15 dram plastic vial without leaving standing water remaining in the bottom of the vial after a 24-hour period (Eliason et al., 2004).

3.3.2 Herbicide Stacking Bioassay Studies

The oriental mustard root bioassay described in Chapter 2 was used to quantify the effect of simultaneous application of imazamox:imazethapyr (1:1) and flucarbazone-sodium on plant toxicity in the four Manitoba soils. For each soil, both the "*expected responses*" and "*observed responses*" were determined. The "*expected response*" is defined as the calculated root inhibition assuming that both imazamox:imazethapyr (1:1) and flucarbazone-sodium are present in soil. The "*observed response*" is defined as the measured root inhibition knowing that both imazamox:imazethapyr (1:1) and flucarbazone-sodium are present in soil. In addition, in order to put the results of the "*expected responses*" and "*observed responses*" in perspective, the results of the "*individual responses*" described in Chapter 2 are also presented in the tables and graphs throughout the current chapter. The "*individual responses*" are thus defined as the measured root inhibition occurring when either imazamox:imazethapyr (1:1) or flucarbazone-sodium are present in soil (i.e. one herbicide treatment). The "*expected responses*" were calculated after Colby (1967):

$$E = X_a Y_b / 100 \qquad [3.1]$$

where E is the expected root length, as a percent of control, when herbicides A and B would have been applied simultaneously at respective a and b dosages, X_a is the measured root length, as a percent of control, when herbicide A was applied individually

at dosage a, and Y_b is the measured root length, as a percent of control, when herbicide B was applied individually at dosage b. Values for X_a and Y_b were taken from the results of the bioassays completed in Chapter 2.

The "*observed responses*" were measured as follows. Stock solutions of each herbicide were prepared by diluting commercial formulations of imazamox:imazethapyr (1:1) (Odyssey[12]) or flucarbazone-sodium (Everest[13]) with deionized water to obtain concentrations of 0.15, 0.30, 0.60, 1.25, 2.50 and 5.00 mg formulated product (f.p.) L^{-1}. Deionized water without herbicides was used as a control (0 mg f.p. L^{-1}). Subsequently, an aliquot (0.75 mL) of each standard solution (herbicide A) was combined with an aliquot (0.75 mL) of either imazamox:imazethapyr (1:1) or flucarbazone-sodium at the 1.25 mg f.p. L^{-1} dosage (herbicide B) in enough distilled water to bring soils to 100 % of their field capacity. Herbicide solutions were mixed with soil in Dixie© cups[14] as described in Chapter 2, yielding the following equivalent application dosages of herbicides A and B, respectively: 0+0 (control), 0+10.5, 1.3+10.5, 2.5+10.5, 5.0+10.5, 10.5+10.5, 21.0+10.5 and 42.0+10.5 mg f.p. m^{+3}. The application rate of 42.0 mg f.p. m^{+3} is approximately equivalent to the field application dosage of 30 g a.i. ha^{+1} for each herbicide, assuming the chemical is evenly distributed in the field through the top 10 cm of soil. As such, these concentrations are here expressed as 0+0 (control), 0+25, 3+25, 6+25, 12+25, 25+25, 50+25 and 100+25 % of the commercial field application dosages of herbicide A and B, respectively. Each treatment was replicated six times. The 25 %

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[12] Odyssey, PCP#25111, 35% + 35% DF formulation, BASF Canada, 100 Milverton Drive, 5th Floor, Mississauga, Ontario Canada, L5R 4H1.

[13] Everest, PCP#26448, 75% DG formulation, Arysta LifeScience North America, 100 First Street, Suite 1700, San Francisco, California USA, 94105.

[14] CC7 Dixie® cups, Georgia-Pacific, Canada Wrap Limited, 196 Sutherland Avenue, Winnipeg, Manitoba Canada, R2W 5K7.

field application dosage of herbicide B was chosen to represent the amount of herbicide that is believed to be an average carry-over residue when either herbicide is applied in the field the previous year (B. Murray, 2005, personal communications). The root bioassay was completed as described in Chapter 2, where pre-germinated oriental mustard seeds were planted into the treated soils, seedling root lengths were measured after 5 days of growth, and the percentages of control were calculated.

In order to establish dose responses for each soil and herbicide(s) combination, data were subjected to nonlinear regression analysis using a 4 parameter log-logistic model (Seefeldt et al., 1995) in SAS version 9.1[15]:

$$y = C + [(D - C) / [1 + \exp[b(\log(x) - \log(I_{50}))]] \quad [3.2]$$

where y = oriental mustard root length expressed as percent of untreated control, x = herbicide dosage expressed as percent of recommended field application dosage (a small positive value of 1.0 was assigned to 0 % dosage to calculate natural logarithms), C = lower limit (asymptote) of the response curve, D = upper asymptote of the response curve, I_{50} = x-axis value that corresponds to the inflection point (i.e. "drop line" to x-axis) and b = slope of the curve at the I_{50} value. For each soil, the "*individual responses*" of oriental mustard to herbicide A without herbicide B; the "*observed response*" of oriental mustard to herbicide A with herbicide B; and the "*expected response*" of herbicide A with herbicide B were statistically tested systematically for common C, common D, common b, and common I_{50} parameter estimates, using the lack-of-fit F test at the 0.05 level of significance as outlined by Seefeldt et al. (1995). When the "*observed response*" and "*expected response*" were equal, the interaction of the two herbicides was considered

[15] SAS version 9.1, 2000, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

additive (i.e. root lengths were similar to that expected). When the "*observed response*" was greater than the "*expected response*", the combination was considered antagonistic (i.e. root lengths were longer than expected). Finally, when the "*observed response*" was less than the "*expected response*", the response from the two herbicides applied was considered synergistic (i.e. root lengths were shorter than expected).

3.4 Results and Discussion

Regardless of whether the herbicides were applied alone or in combination, the response of oriental mustard root length to increasing dosages of imazamox:imazethapyr (1:1) and flucarbazone-sodium applied was described very well by the log-logistic model (Tables 3.1 and 3.2).

3.4.1 Observed "*Individual Responses*" of Herbicides (taken from Chapter 2)

The results of the bioassay experiments with imazamox:imazethapyr (1:1) or flucarbazone-sodium applied individually to soil are discussed in Chapter 2. Although the same data from Chapter 2 was used for Chapter 3, the *"Individual Response"* parameter estimates reported in Chapter 3 differ slightly from those reported in Chapter 2 (Table 2.2, Table 3.1, Table 3.2). This occurs because the lack-of-fit *F* test (described above) was applied to different sets of curves in each of Chapter 2 and 3. In Chapter 2, for each herbicide individual curves for each soil type were statistically tested systematically for common parameter estimates (Table 2.2), while in Chapter 3, the "*individual responses"* of oriental mustard to herbicide A without herbicide B; the "*observed response*" of oriental mustard to herbicide A with herbicide B; and the

"*expected response*" of herbicide A with herbicide B were statistically tested systematically for common parameter estimates (Table 3.1, Table 3.2).

Table 3.1 Equations describing the response of oriental mustard root length to increasing dosages of flucarbazone-sodium (as a percentage of recommended field dosage) in four Manitoba soils treated with or without imazamox:imazethapyr (1:1) at 25 % of the recommended application dosage. Parameter estimates (C, D and b) are for root lengths expressed as a percentage of untreated controls ± standard errors.

Soil Series	Treatment	C[a] ± SE	D ± SE	b ± SE	I_{50}[b] ± SE
Lundar CL	Individual Response[c]	17.2 ± 1.6	97.2 ± 1.0	2.00 ± 0.14	30.3 ± 1.1
	Observed Response[d]	17.2 ± 1.6	42.9 ± 0.7	0.94 ± 0.18	62.0 ± 12.9
	Expected Response[e]	7.4 ± 1.1	42.9 ± 0.7	2.00 ± 0.14	30.3 ± 1.1
Manitou SL	Individual Response	7.2 ± 2.4	98.3 ± 1.6	3.03 ± 0.39	64.3 ± 3.5
	Observed Response	7.2 ± 2.4	35.7 ± 1.3	0.99 ± 0.34	64.3 ± 3.5
	Expected Response	7.2 ± 2.4	35.7 ± 1.3	3.03 ± 0.39	64.3 ± 3.5
Red River C	Individual Response	18.6 ± 0.9	99.4 ± 0.7	3.51 ± 0.21	40.3 ± 0.8
	Observed Response	18.6 ± 0.9	40.7 ± 0.5	1.60 ± 0.29	40.3 ± 0.8
	Expected Response	7.2 ± 0.9	40.7 ± 0.5	3.51 ± 0.21	40.3 ± 0.8
Stockton LS	Individual Response	11.5 ± 1.7	97.9 ± 1.6	2.09 ± 0.18	17.5 ± 0.8
	Observed Response	3.0 ± 1.2	23.4 ± 0.8	2.09 ± 0.18	109.1 ± 20.5
	Expected Response	3.0 ± 1.2	23.4 ± 0.8	2.09 ± 0.18	17.5 ± 0.8

[a] Statistical differences between parameter estimates were determined using the lack-of-fit F test at the 0.05 level of significance (refer to Materials and Methods). R^2 values for all soil series were 0.99.

[b] I_{50} values are percentages of recommended field application dosages.

[c] "*Individual Response*" values are of root lengths observed when only flucarbazone-sodium was present in the soil.

[d] "*Observed Response*" values are of root lengths observed when both flucarbazone-sodium at increasing dosages and imazamox:imazethapyr (1:1) at the 25 % dosage were present in the soil.

[d] "*Expected Response*" values were calculated following Colby (1967).

Table 3.2 Equations describing the response of oriental mustard root length to increasing dosages of imazamox:imazethapyr (1:1) (as a percentage of recommended field dosage) in four Manitoba soils treated with or without flucarbazone-sodium at 25% of the recommended application dosage. Parameter estimates (C, D and b) are for root lengths expressed as a percentage of untreated controls ± standard errors.

Soil Series	Treatment	C[a] ± SE	D ± SE	b ± SE	I_{50}[b] ± SE
Lundar CL	Individual Response[c]	12.5 ± 2.8	120.0	0.76 ± 0.06	7.3 ± 0.8
	Observed Response[d]	12.5 ± 2.8	94.2 ± 5.6	0.62 ± 0.07	5.1 ± 1.2
	Expected Response[e]	12.5 ± 2.8	94.2 ± 5.6	0.76 ± 0.06	5.1 ± 1.2
	Bound (*D_Individual Response*)		0.1 ± 0.7		
Manitou SL	Individual Response	18.6 ± 1.1	102.5 ± 2.0	2.10 ± 0.18	13.6 ± 0.7
	Observed Response	18.6 ± 1.1	83.2 ± 1.7	2.10 ± 0.18	5.5 ± 0.4
	Expected Response	18.6 ± 1.1	83.2 ± 1.7	2.10 ± 0.18	13.6 ± 0.7
Red River C	Individual Response	21.1 ± 1.2	101.7 ± 1.7	1.77 ± 0.13	15.7 ± 0.8
	Observed Response	21.1 ± 1.2	112.0	0.98 ± 0.07	3.2 ± 0.2
	Expected Response	21.1 ± 1.2	94.1 ± 1.7	1.77 ± 0.13	15.7 ± 0.8
	Bound (*D_Observed Response*)		1.0 ± 0.4		
Stockton LS	Individual Response	14.3 ± 1.1	109.3 ± 4.1	1.68 ± 0.17	4.0 ± 0.3
	Observed Response	14.3 ± 1.1	47.8 ± 2.0	0.94 ± 0.17	4.0 ± 0.3
	Expected Response	6.1 ± 1.0	47.8 ± 2.0	1.68 ± 0.17	4.0 ± 0.3

[a] Statistical differences between parameter estimates were determined using the lack-of-fit F test at the 0.05 level of significance (refer to Materials and Methods). R^2 values for all soil series were 0.99.

[b] I_{50} values are percentages of recommended field application dosages.

[c] "*Individual Response*" values are of root lengths observed when only imazamox:imazethapyr (1:1) was present in the soil.

[d] "*Observed Response*" values are of root lengths observed when both imazamox:imazethapyr (1:1) at increasing dosages and flucarbazone-sodium at the 25 % dosage were present in the soil.

[d] "*Expected Response*" values were calculated following Colby (1967).

3.4.2 *"Observed Responses"* and *"Expected Responses"* of Herbicides Applied Simultaneously

The upper asymptotes (*D*) were significantly higher when flucarbazone-sodium was applied alone ("*individual response*") than when flucarbazone-sodium was combined with imazamox:imazethapyr (1:1) at 25 % of its field application dosage ("*observed response*") (Table 3.1, Figure 3.1). Smaller significant differences were observed between upper asymptotes (*D*) of soils containing imazamox:imazethapyr (1:1) alone ("*individual response*") versus imazamox:imazethapyr (1:1) in combination with flucarbazone-sodium residues at 25 % of its recommended field dosage ("*observed response*") (Table 3.2, Figure 3.2). Thus, the imazamox:imazethapyr (1:1) residues caused a greater decrease in root lengths than the flucarbazone-sodium residues, as is expected since oriental mustard is more sensitive to imazamox:imazethapyr (1:1) than to flucarbazone-sodium (Chapter 2). The exception to this was the coarse-textured Stockton LS, which demonstrated relatively large differences between upper asymptotes of soils containing flucarbazone-sodium only and soils containing both flucarbazone-sodium and imazamox:imazethapyr (1:1) (Figure 3.1) and between upper asymptotes of soils containing imazamox:imazethapyr (1:1) only and soils containing both imazamox:imazethapyr (1:1) and flucarbazone-sodium (Figure 3.2). This is likely due to the lesser ability of the Stockton LS to sorb increasing herbicide residues (Chapter 2), making them more bioavailable to be phytotoxic.

As the dosage of flucarbazone-sodium increased in soils containing imazamox:imazethapyr (1:1) residues (Table 3.1, Figure 3.1), the corresponding reduction in root length was not as great as that in soils containing flucarbazone-sodium

residues and receiving increasing dosages of imazamox:imazethapyr (1:1) (Table 3.2, Figure 3.2). Again, this was likely due to the greater phytotoxicity of imazamox: imazethapyr (1:1) to oriental mustard.

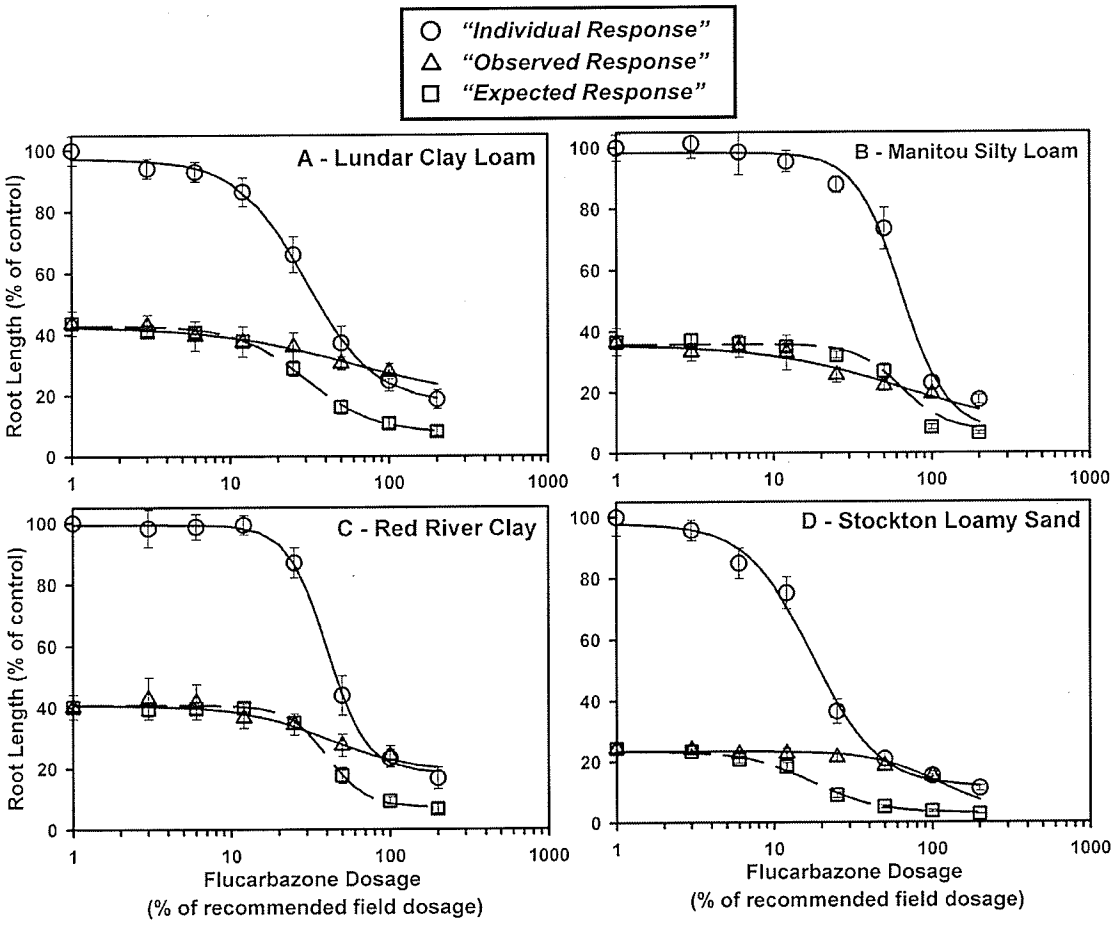

Figure 3.1 ***"Individual", "observed" and "expected"*** **(as calculated following Colby (1967)) dose response curves of oriental mustard root lengths (% of untreated control) grown in four Manitoba soils (A-D) containing either flucarbazone-sodium alone (*"individual"*) or both flucarbazone-sodium (at a range of dosages) and imazamox:imazethapyr (1:1) (at 25 % of recommended field dosage) (*"observed"* and *"expected"*). Symbols are the means ± standard deviations. Refer to Table 3.1 for parameter estimates of the log-logistic model fitted. Curves were significantly different according to the lack-of-fit F test (refer to Materials and Methods).**

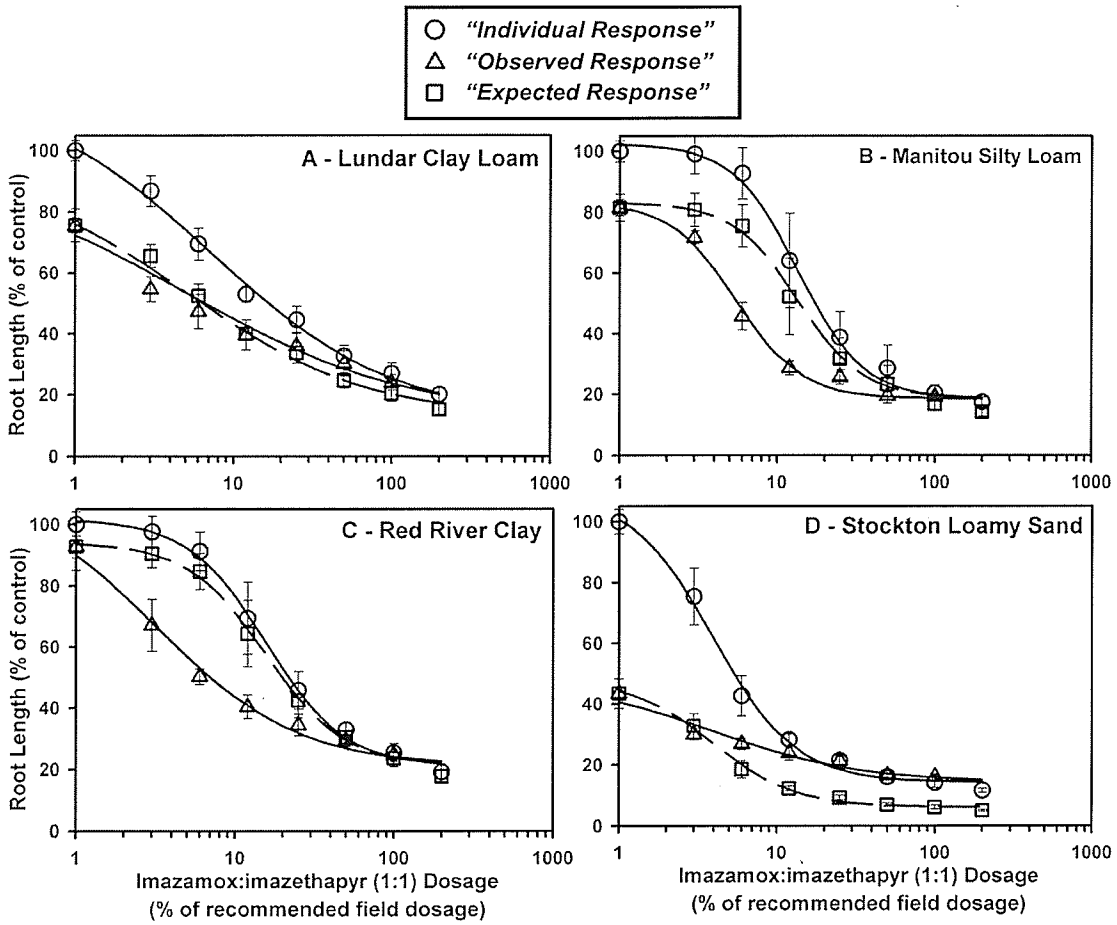

Figure 3.2 ***"Individual", "observed" and "expected"*** **(as calculated following Colby (1967)) dose response curves of oriental mustard root lengths (% of untreated control) grown in four Manitoba soils (A-D) containing either imazamox:imazethapyr (1:1) alone (*"individual"*) or both imazamox: imazethapyr (1:1) (at a range of dosages) and flucarbazone-sodium (at 25 % of recommended field dosage) (*"observed"* and *"expected"*). Symbols are the means ± standard deviations. Refer to Table 3.2 for parameter estimates of the log-logistic model fitted. Curves were significantly different according to the lack-of-fit F test (refer to Materials and Methods).**

3.4.2.1 Flucarbazone-sodium Application to Soils Containing Imazamox: imazethapyr (1:1) Residues. In the Manitou SL, Red River C and Lundar CL soils, the slopes (*b*) of curves of "*expected responses*" were significantly greater than the slopes of the curves of "*observed responses*" (Table 3.1, Figure 3.1). Thus, when imazamox:imazethapyr (1:1) residues (25 % of recommended field dosage) were present in the soil, the addition of increasing dosages of flucarbazone-sodium had a less harmful effect on oriental mustard root length than expected. It is possible that when both herbicides were present in the soil, the effect of the imazamox:imazethapyr (1:1) residue masked the effect of increasing dosages of flucarbazone-sodium, due to its greater phytotoxicity to oriental mustard. Slopes of "*observed responses*" and "*expected responses*" in the Stockton LS were the same, indicating the addition of increasing dosages of flucarbazone-sodium affected the oriental mustard as expected, again likely due to the always low sorptive capacity of the soil.

In both the Manitou SL and Red River C, the I_{50} values in the "*observed responses*" and "*expected responses*" were not significantly different, indicating an additive effect at the I_{50} dosage (Table 3.1, Figure 3.1). In contrast, the I_{50} values in the Lundar CL and Stockton LS were higher in the "*observed*" than in the "*expected*" curves, indicative of antagonistic interactions.

The lower asymptotes (*C*) of the Lundar CL and Red River C soils were greater in the "*observed*" than in the "*expected*" curves, indicating antagonistic effects at higher dosages (Table 3.1, Figure 3.1). In the Manitou SL and Stockton LS series, lower asymptotes were not significantly different in the "*observed*" and "*expected*" curves, so the interaction was additive at corresponding dosages.

Hence, results demonstrated that depending on soil characteristics and the amounts of herbicide residues in soil, the effects were either additive or antagonistic. To provide an example representing a practical field situation, a producer would apply flucarbazone-sodium at the 100 % field application dosage to a soil containing imazamox:imazethapyr (1:1) residues. In soils such as the Lundar CL, Red River C and Stockton LS, lesser crop damage than expected would result (i.e. antagonism) and hence stacking as defined by Johnson et al. (2005) will not be noted (Table 3.1, Figure 3.1). Damage similar to that expected would occur in the Manitou SL series (i.e. additive interaction), and thus, as defined by Johnson et al. (2005), stacking would be a possibility. The results for the Manitou SL series agree well with Johnson et al. (2005) who observed both additive and synergistic responses where imazamox:imazethapyr (1:1) had been applied in the year previous to a flucarbazone-sodium application on a loam soil with low organic matter (3 %) and low pH (5.9) in the brown soil zone of Saskatchewan.

3.4.2.2 Imazamox:imazethapyr (1:1) Application to Soils Containing Flucarbazone-sodium Residues. For Red River C and Stockton LS, the slopes (*b*) of curves of expected responses were significantly greater than the slopes of the curves of "*observed*" responses (Table 3.2, Figure 3.2). Thus, when flucarbazone-sodium residues (25 % of recommended field dosage) were present in the soil, the addition of increasing dosages of imazamox:imazethapyr (1:1) had a less harmful effect than expected on oriental mustard root length. This is similar to what was seen in soils containing imazamox:imazethapyr (1:1) residues (Section 3.4.2.1).

In both the Lundar CL and Stockton LS, the I_{50} values of the "*observed*" and "*expected*" responses were not significantly different, indicating an additive effect at the I_{50} dosage (Table 3.2). In contrast, the I_{50} values of the Manitou SL and Red River C were lower in the "*observed*" than in the "*expected*" curves, indicative of a synergistic interaction. A similar result was observed by Johnson et al. (2005) on a loam soil in the brown soil zone of western Saskatchewan, when a flucarbazone-sodium application was made the year following imazamox:imazethapyr (1:1) use. They attributed this synergism to the low pH, low organic matter, and low growing season precipitation at the site, allowing for greater carry-over of the imazamox:imazethapyr (1:1) into the year when flucarbazone-sodium was applied. In our experiment, soils were maintained at 100 % of their field capacity, and carry-over was only simulated, as both herbicides were applied simultaneously. It is possible, however, that due to its low soil pH, there are larger amounts of imazamox:imazethapyr (1:1) in the water phase of the Manitou SL soil. For example, Bresnahan et al. (2002; 2000) reported that although imazamox and imazethapyr will sorb more at pH < 6, they are more readily desorbed under acidic conditions, whereas in alkaline soils, although sorption is low, those herbicides that do sorb, are less likely to desorb. Thus, with the ideal moisture conditions of the acidic Manitou SL soil, it is possible that the imazamox:imazethapyr (1:1) was readily desorbed from the soil and was replaced by the less phytotoxic flucarbazone-sodium. This could reduce any further sorption of the imazamox:imazethapyr (1:1), making it more bioavailable and causing the synergistic response.

The lower asymptotes (C) of Stockton LS were greater in the observed than in the expected curves, indicating antagonistic effects (Table 3.2, Figure 3.2). In contrast, the

lower asymptotes were not significantly different in the observed and expected curves of the remaining three soils, so the interaction was additive in those soils at higher dosages.

Thus, in a field situation where imazamox:imazethapyr (1:1) is applied at its field application dosage (100 %) to soils such as Lundar CL, Manitou SL and Red River C containing flucarbazone-sodium residues, crop damage similar to expected could result (i.e. additive interaction) (Figure 3.2), and hence "stacking" as defined by Johnson et al. (2005) occurs. Interestingly, Geisel et al. (2008) also observed additive responses in three field experiments conducted on clay loam, silt clay and loam soils in central Saskatchewan, where two ALS-inhibiting herbicides (including imazamox:imazethapyr (1:1) and flucarbazone-sodium) were sequentially applied over the course of two years. Current results indicate that damage less than expected would only occur in the Stockton LS (i.e. antagonistic interaction).

3.5 Conclusions

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are Group 2 (ALS-Inhibitor) herbicides whose persistence in soil can lead to crop damage in years following their application. Imazamox:imazethapyr (1:1) and flucarbazone-sodium phytotoxicity interactions were assessed in four Manitoba soils using the oriental mustard root bioassay procedure, by comparing "*expected responses*" and "*observed responses*" of the herbicide mixtures. When imazamox:imazethapyr (1:1) carry-over was simulated, and increasing rates of flucarbazone-sodium were applied, both antagonistic and additive responses were observed at field application dosages of flucarbazone. When the herbicides were applied to simulate flucarbazone-sodium carry-over, synergistic

interaction responses were observed in the Manitou silt loam and Red River clay soil series at intermediate dosages of imazamox:imazethapyr (1:1), while both additive and antagonistic results were observed at field application dosages of imazamox:imazethapyr (1:1). Thus, there is potential for "stacking" (i.e. increased adverse effects) in some Manitoba soils with back-to-back application of certain ALS inhibitor herbicides.

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4. DISSIPATION OF IMAZAMOX:IMAZETHAPYR (1:1) AND FLUCARBAZONE-SODIUM IN MANITOBA SOILS AS A FUNCTION OF SOIL MOISTURE CONTENT, TEMPERATURE AND NUTRIENT LEVELS.

4.1 Abstract

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are ALS inhibitor (Group 2) herbicides containing active ingredients that have a strong potential to persist in soil. These herbicide residues may damage subsequent sensitive crops when they are bioavailable to the plant by root uptake. Since there are limited studies on the persistence of imazamox:imazethapyr (1:1) and flucarbazone-sodium in Manitoba soils, this study applied an oriental mustard root bioassay to four Manitoba soil series ranging in texture from clay to sandy loam spiked with either imazamox:imazethapyr (1:1) or flucarbazone-sodium. Soils were spiked with either herbicide at 100 % of their commercial field rates and then incubated for 16 weeks at varying moisture, temperature and nitrogen levels, and the bioassay was conducted at 0, 1, 2, 4, 8 and 16 weeks incubation. Root lengths (expressed as a percent of untreated control) were reported for each incubation. Generally, average root lengths were longer in flucarbazone-sodium-treated soils as compared to imazamox:imazethapyr (1:1)-treated soils. Root lengths increased with increasing soil moisture from 50 to 75 to 100 % field capacity for both herbicides due to increasing degradation, however flucarbazone-sodium bioavailability was found to be less affected by decreasing moisture, as compared to imazamox:imazethapyr (1:1). Differences observed in soils for imazamox:imazethapyr (1:1) are based on their sorption and desorption capacities (particularly in soils with low pH) and the microbial activity of

the soil. Response in flucarbazone-sodium-treated soils was more affected by declining temperature (25 to 15 to 5°C) than in imazamox: imazethapyr (1:1)-treated soils, however both herbicides were quite stable at the lowest temperature, indicative of low microbial degradation. Differences between the nitrogen treatments were minimal in soils containing flucarbazone-sodium, and more pronounced in soils containing imazamox:imazethapyr (1:1), where phytotoxicity increased with increasing soil nitrogen.

4.2 Introduction

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are ALS inhibitor (Group 2) herbicides frequently used in western Canadian agriculture. Imazamox:imazethapyr (1:1) are imidazolinones applied post emergence to field peas, soybeans, alfalfa and imidazolinone-tolerant (Clearfield) canola and lentils to control both grassy and broadleaf weeds (Anonymous, 2009a; Vencill, 2002a; Vencill, 2002b). Flucarbazone-sodium is a relatively new post emergence herbicide, classified as a sulfonylaminocarbonyl-triazolinone, used to control grassy and some broadleaf weeds in spring wheat and durum (Anonymous, 2009b; Vencill, 2002c).

Group 2 herbicides, including active ingredients imazethapyr, imazamox and flucarbazone-sodium, have a strong potential to persist in soil past the season of application, potentially damaging subsequent sensitive crops (Jourdan et al., 1998a; Loux et al., 1989; Moyer and Esau, 1996; O'Sullivan et al., 1998). Herbicide residues in soil can be phytotoxic when they are bioavailable to the plant by root uptake, and this herbicide bioavailability is influenced by soil chemical and physical properties (Eliason

et al., 2004; Ortega et al., 2004; Williams et al., 2002). Bioassays are sensitive, simple techniques that can estimate bioavailable herbicide residues in soil and aid in understanding the relation between soil properties and herbicide phytotoxicity over time. Eliason et al. (2004) tested various crops to determine which could best provide a sensitive, accurate bioassay for the detection of flucarbazone-sodium in soil. Of the five crops they tested, oriental mustard (*Brassica juncea*) root length was found to be the best indicator. Eliason et al. (2004) measured flucarbazone-sodium phytotoxicity and persistence in five Saskatchewan soils and one Manitoba soil, and found that its half-life was significantly greater in the Manitoba soil.

Herbicide bioavailability over time (i.e. persistence) can be affected by soil properties and environmental factors, including soil moisture content, temperature and nutrient levels. According to the Manitoba Agriculture Yearbook, fertilizer has consistently been one of the largest farm operating expenditures in Manitoba, with 943,200 tonnes sold in 2004 (Anonymous, 2004). Of the fertilizer inputs applied, nitrogen (in various formulations) makes up the largest segment of fertilizer sales, at over 55 % in 2004 (Anonymous, 2004). Nitrogen levels have been shown to influence the susceptibility of plants to herbicides, however results differ based on plant species and herbicides applied (Cathcart et al., 2004; Chao et al., 1994; Lutman et al., 1975). Although studies have been completed regarding the persistence of imazamox and imazethapyr in soil, little work has been done on flucarbazone-sodium, and no work has been conducted on the influence of soil nitrogen applications on the persistence of flucarbazone-sodium or imazamox:imazethapyr (1:1) herbicides in Manitoba soils.

The objective of this study was to use the oriental mustard root bioassay (Eliason et al. (2004) to quantify the effects of soil properties, soil moisture, soil temperature and ammonium nitrate application on the bioavailability of imazamox:imazethapyr (1:1) and flucarbazone-sodium over time in Manitoba soils.

4.3 Methods

4.3.1 Soil Series and Properties

Four surface soils (0-10 cm), with varying properties and no history of imazamox:imazethapyr (1:1) or flucarbazone-sodium application were collected from across southern Manitoba as described in Chapter 2. Soils are here identified by their soil series classification and soil texture: Lundar Clay Loam (Lundar CL), Manitou Silt Loam (Manitou SL), Red River Clay (Red River C) and Stockton Loamy Sand (Stockton LS). Soils were air-dried and sieved (< 2 mm) prior to soil property and bioassay analyses.

As described in Chapter 2, soil organic carbon content (SOC) was determined by dry combustion (Nelson and Sommers, 1982); soil texture was measured using the hydrometer method (Gee and Bauder, 1986); soil pH was determined with $CaCl_2$ (Hendershot and Lalande, 1993); and NO_3-N, SO_4-S, P and K were quantified using the automated Cadmium Reduction Method 4500-NO_3, the Turbidimetric Method 4500-SO_4^{2-}, the Stanneous Chloride Method 4500-P and the Flame Photometric Method 3500-K, respectively (Clesceri et al., 1998). Field capacity (as a percent) was measured by determining the weight of water required to completely wet a 35 g sample of air-dried soil to the bottom of a 15 dram plastic vial without leaving standing water remaining in the bottom of the vial after a 24-hour period (Eliason et al., 2004).

In addition, the fluorescein diacetate hydrolosis assay (FDA) (Adam and Duncan, 2001) was used to measure the total microbial activity in the four soils. Soils were brought to 100 % field capacity with distilled water in Dixie© cups[16] and duplicate 2 g samples of soil (dry weight basis) were removed for FDA analysis. The remaining soil was incubated at 25°C and soils were sub sampled (2 g on a dry weight basis) at 24 hours and at 14 days. During incubation, soils were watered to maintain 100 % field capacity every 5 days for Lundar SL, Manitou SL and Red River C, and every 3 days for Stockton LS to prevent moisture losses in excess of 10 % (by weight). For each sample, the amount of fluorescein in the sample filtrate was measured at 490 nm on a spectrophotometer (Biochrom Ultrospec 3100 pro, Cambridge, UK), and total microbial activity was measured as the amount of fluorescein hydrolysed.

FDA data was tested for normality using the KRUSKAL-WALLIS test in SAS version 9.1[17], and was found to be normally distributed. Statistical analyses were done to test for the impact of soil properties and incubation time on microbial activity using a two-way ANOVA in SigmaStat version 3.5[18] with factors soil (Lundar CL, Manitou SL, Red River C, Stockton LS) and incubation time (0, 1, 14 days), followed by a Tukey's multiple comparison test with a significance level of $P \leq 0.05$.

4.3.2 Oriental Mustard Root Bioassay

The oriental mustard root bioassay described in Chapter 2 was adapted to study the effect of soil properties, moisture, temperature and nitrogen rates on the persistence of imazamox:imazethapyr (1:1) and flucarbazone-sodium in the Manitoba soils. For each

[16] CC7 Dixie® cups, Georgia-Pacific, Canada Wrap Limited, 196 Sutherland Avenue, Winnipeg, Manitoba Canada, R2W 5K7.
[17] SAS version 9.1, 2000, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.
[18] SigmaStat version 3.5 for Windows, 2006, Systat Software Inc., Chicago, IL 60606.

herbicide (either imazamox:imazethapyr (1:1) or flucarbazone-sodium), treatments included three nitrogen levels in the nitrogen experiment, three moisture levels in the moisture experiment and three temperature levels in the temperature experiment, as follows:

- *Moisture experiment:* 50, 75 or 100 % field capacity at 25°C without nitrogen additions.
- *Temperature experiment:* 5, 15 or 25°C at 100 % field capacity without nitrogen additions.
- *Nitrogen experiment:* 0, 75 or 150 kg N ha^{-1} at 100 % field capacity and 25°C.

The effects of moisture were examined for the four Manitoba soils, while the effects of temperature and nitrogen rates were examined for the Lundar CL and Stockton LS only, reflecting the two soils with the strongest differences in soil characteristics (Table 2.1). Therefore, in total 1,440 cups of soil were set up in this study, 720 cups for imazamox:imazethapyr (1:1) and 720 cups for flucarbazone-sodium testing. For each herbicide, the numbers of pots in the three different experiments were:

- *Moisture experiment:* 3 levels X 4 soils X 5 sampling weeks X 6 replicates = 360
- *Temperature experiment:* 3 levels X 2 soils X 5 sampling weeks X 6 replicates = 180
- *Nitrogen experiment:* 3 levels X 2 soils X 5 sampling weeks X 6 replicates = 180

For the nitrogen experiment, soils were spiked with nitrogen and allowed to incubate for two weeks prior to herbicide application. An ammonium nitrate stock solution with a concentration of 25.5 g NH_4NO_3 L^{-1} (or 8.9 g N L^{-1}) was prepared in deionized water. As described in Chapter 2, aliquots (0.75 and 1.5 mL, respectively) of the ammonium nitrate stock solution were added to enough distilled water required to

bring the soils to 75 % of their field capacities and then mixed with soil to establish concentrations of 214.3 and 428.6 g NH_4NO_3 m^{-3} (i.e. 75 and 150 kg N ha^{-1}assuming the nitrogen is evenly distributed through the top 10 cm of soil). The treatment without ammonium nitrate additions (0 kg N ha^{-1}) was the control treatment. Each cup of soil was covered with a Dixie® domed lid[19] with a 5 mm hole drilled in the centre, and incubated at 25°C for two weeks to allow for the processes of nitrification and denitrification to occur before herbicide application. During incubation, cups of soil were watered (by weight) to 75 % field capacity when not more than 10 % moisture loss occurred (every 3 days for Stockton LS and 5 days for Lundar CL). Additional bulk cups of Lundar CL and Stockton LS were set up with ammonium nitrate (0, 75 and 100 kg N ha^{-1}) and maintained at 75 % field capacity, as described above. Duplicate 2 g samples were taken after 24 hours and 14 days incubation and used in the FDA assay as described above. FDA data was tested for normality of distribution using the KRUSKAL-WALLIS test in SAS version 9.1, and was found to be normally distributed. Statistical analyses were done to test for the impact of soil properties and nitrogen levels on microbial activity using two-way ANOVA for soil (Ld and St) and nitrogen level (0, 75 and 100 kg N ha^{-1}) in SigmaStat version 3.5. Duration of incubation (24 hours and 14 days) was previously determined not to have a significant effect, thus data were combined over the incubation times for ANOVA analysis. Mean comparison was completed using Tukey's multiple comparison test with a significance level of $P \leq 0.05$.

[19] DF57 Dixie® domed lid, Georgia-Pacific, Canada Wrap Limited, 196 Sutherland Avenue, Winnipeg, Manitoba Canada, R2W 5K7.

Stock solutions of each herbicide were prepared by diluting commercial formulations of imazamox:imazethapyr (1:1) (Odyssey[20]) or flucarbazone-sodium (Everest[21]) with deionized water to obtain a concentration of 5.00 mg formulated product (f.p.) L^{-1}. For the moisture experiment, an aliquot (0.75 mL) of either imazamox:imazethapyr (1:1) or flucarbazone-sodium was added to enough distilled water to bring the four soils to 50, 75 or 100 % of their field capacity. For the nitrogen and temperature experiments, the same aliquot (0.75 mL) was added to enough distilled water to bring the two soils to 100 % of their field capacity. Solutions were mixed in soil measured into Dixie© cups (as described in Chapter 2), yielding an application dosage of 42.0 mg f.p. m^3. The application rate of 42.0 mg f.p. m^3 is approximately equivalent to the field application dosage of 30 g a.i. ha^1 for each herbicide, assuming the chemical is evenly distributed through the top 10 cm of soil. As such, these concentrations are here expressed as 100 % of the commercial field application dosages of either imazamox:imazethapyr (1:1) or flucarbazone-sodium.

Each cup of soil was covered with a Dixie® domed lid with a 5 mm hole drilled in the centre to allow for gas diffusion, and incubated for 1, 2, 4, 8 or 16 weeks. During incubation, cups of soil were watered (by weight) to the required field capacity when not more than 10 % moisture loss occurred (every 3 days for Stockton LS and 5 days for the remaining three soils). At 1, 2, 4, 8 or 16 weeks, one set of replicates from each treatment was removed from the incubator (i.e. six cups of soil per treatment). The soil was brought up to 100 % field capacity, thoroughly mixed, and the root bioassay was

[20] Odyssey, PCP#25111, 35% + 35% DF formulation, BASF Canada, 100 Milverton Drive, 5th Floor, Mississauga, Ontario Canada, L5R 4H1.

[21] Everest, PCP#26448, 75% DG formulation, Arysta LifeScience North America, 100 First Street, Suite 1700, San Francisco, California USA, 94105.

completed as described in Chapter 2, where pre-germinated oriental mustard seeds were planted into the treated soils, seedling root length was measured after 5 days of growth, and percent of control was calculated. Root length data for week 0 was taken from corresponding bioassay experiments described in Chapter 2.

Root length data, expressed as a percent of control was tested for normality of distribution using PROC UNIVARIATE in SAS version 9.1. Root length data was found not to be normally distributed; therefore it was normalized by natural log transformation prior to analysis. For each herbicide (imazamox:imazethapyr (1:1) or flucarbazone-sodium), data were analyzed separately for each factor (i.e. Moisture, Nitrogen, or Temperature). For each separate analysis, PROC MIXED for REPEATED MEASURES was used with time as the repeated effect, and soil and the level of moisture or nitrogen or temperature as the fixed effects; replicates and interactions involving replications were random effects. Spatial power [SP(POW)] covariance structure was used in the models since time intervals were unequal and other structures could therefore not be used. (NB: SP(POW) for unequally spaced data provides a direct generalization of the first order autoregressive [AR(1)] structure for equally spaced data.) Mixed-model F tests, based on the Kenward–Roger (Kenward and Roger, 1997) adjusted denominator degrees of freedom approximation, were used to assess the significance of fixed effects and interactions. Mean comparison was completed using Tukey's multiple comparison test, with a significance level of $P \leq 0.05$. Following analysis, the inverse of the natural logarithm means and standard errors was taken for presentations in tables.

In order to estimate the bioavailable herbicide concentration remaining in the soil at 0, 1, 2, 4, 8 or 16 weeks, the herbicide dosage corresponding to the observed root

lengths (expressed as a percent of control) was calculated using the 4 parameter log-logistic model developed by Seefeldt et al. (1995), with parameters solved for in Chapter 2 (Tables 2.2 and 2.5):

$$x = I_{50} [((D - C) / (y - C) - 1)^{(1/b)}] \quad [4.1]$$

where x = herbicide dosage (expressed as percent of recommended field application dosage), y = oriental mustard root length (expressed as percent of untreated control), C = lower limit (asymptote) of the response curve, D = upper asymptote of the response curve, I_{50} = x-axis value that corresponds to the inflection point (i.e. "drop line" to x-axis) and b = slope of the curve at the I_{50} value. Calculated herbicide dosages above 100 % of the field application rate were adjusted to 100 %, as this was the maximum dosage applied to the soil, and any values less than 0 % were adjusted to 0 %.

4.4 Results and Discussion

4.4.1 Microbial activity

Total microbial activity, as measured using the fluorescein diacetate hydrolosis assay, was found not to be significantly affected by duration of incubation (P=0.681) in analyses of soils (Lundar CL, Manitou SL, Red River C, Stockton LS) incubated at 100 % field capacity. Soil, however, did significantly affect microbial activity (P=0.012), with Red River C having the lowest activity of the four soils. This may be attributed to the high clay content of the Red River C, and/or the soil's higher nitrogen content (Table 2.1). Muller and Hoper (2004) found a positive correlation between soil clay content and soil microbial biomass, but a negative correlation between soil clay content and metabolic quotient, an indicator of specific microbial activity. Although there have been

inconsistent results linking soil nitrogen and soil microbial biomass, some researchers have found that soils fertilized with nitrogen show low soil CO_2 emissions (i.e. low microbial activity) (Kowalenko et al., 1978). Since duration of incubation did not have a significant effect, soils (Lundar CL, Stockton LS) incubated at 75 % field capacity with the addition of nitrogen were analyzed for all incubation times combined for soil and nitrogen effects. Soil was found to be the only significant factor (soil P=0.012, nitrogen P=0.941), where the microbial activity was significantly greater in the Lundar CL than Stockton LS, as expected because of the low organic carbon content of the Stockton LS (Table 2.1). Although not significant, when averaged over the two soils, increasing nitrogen content did numerically decrease microbial activity, which agrees with Kowalenko et al. (1978).

4.4.2 Imazamox:Imazethapyr (1:1) and Flucarbazone-Sodium Dissipation

In comparing the three experiments (moisture, temperature, nitrogen), it is important to note that the curves of soils incubated at 100 % field capacity, 25°C and with no nitrogen (M100: Figure 4.1 and 4.2; T25: Figure 4.3 and 4.4; N0: Figure 4.5 and 4.6) are similar for each of the herbicide treatments. Since these experiments were conducted separately (with the same parameters), this indicates that the results of the experiments are reproducible.

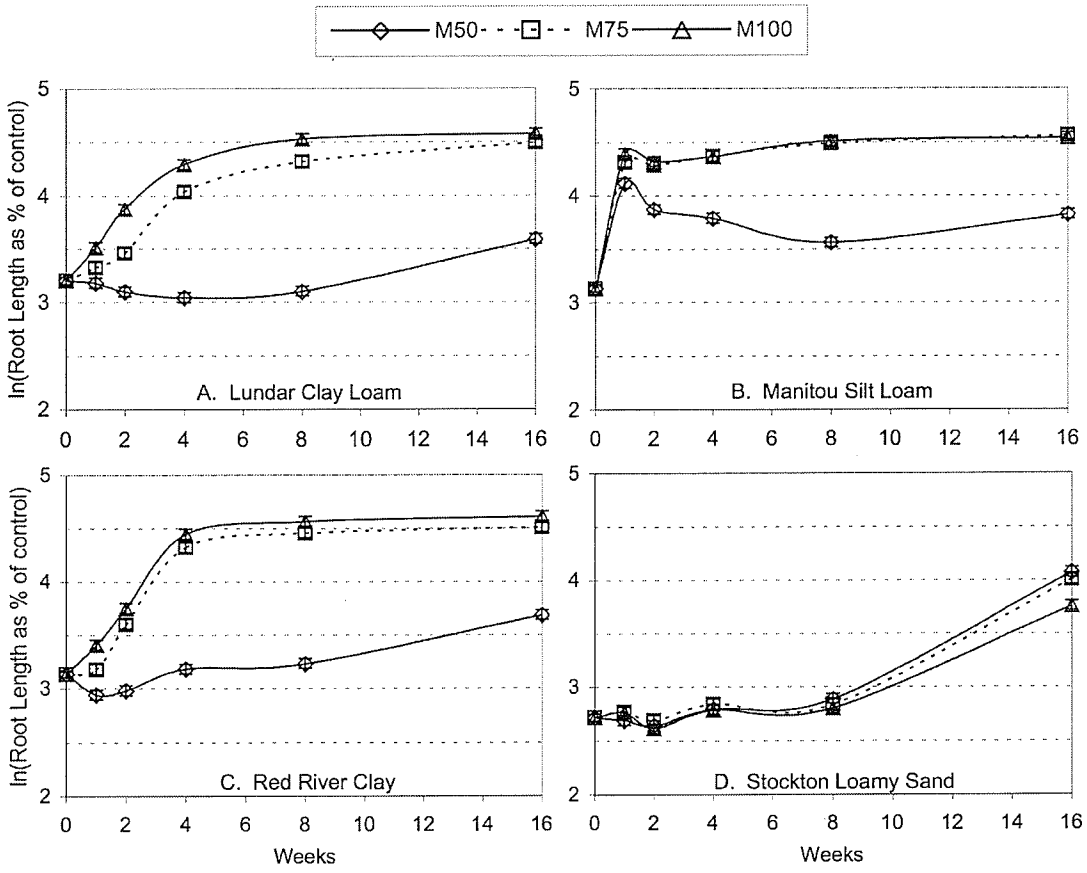

Figure 4.1 Effects of soil properties (A, B, C, D), moisture levels (M50 = 50 % field capacity, M75 = 75 % field capacity, M100 = 100 % field capacity) and duration of incubation (weeks) on oriental mustard root lengths (expressed as the natural logarithm of percent of control) grown in soils treated with Imazamox:Imazethapyr (1:1) at 100 % of its field application dosage on day 0.

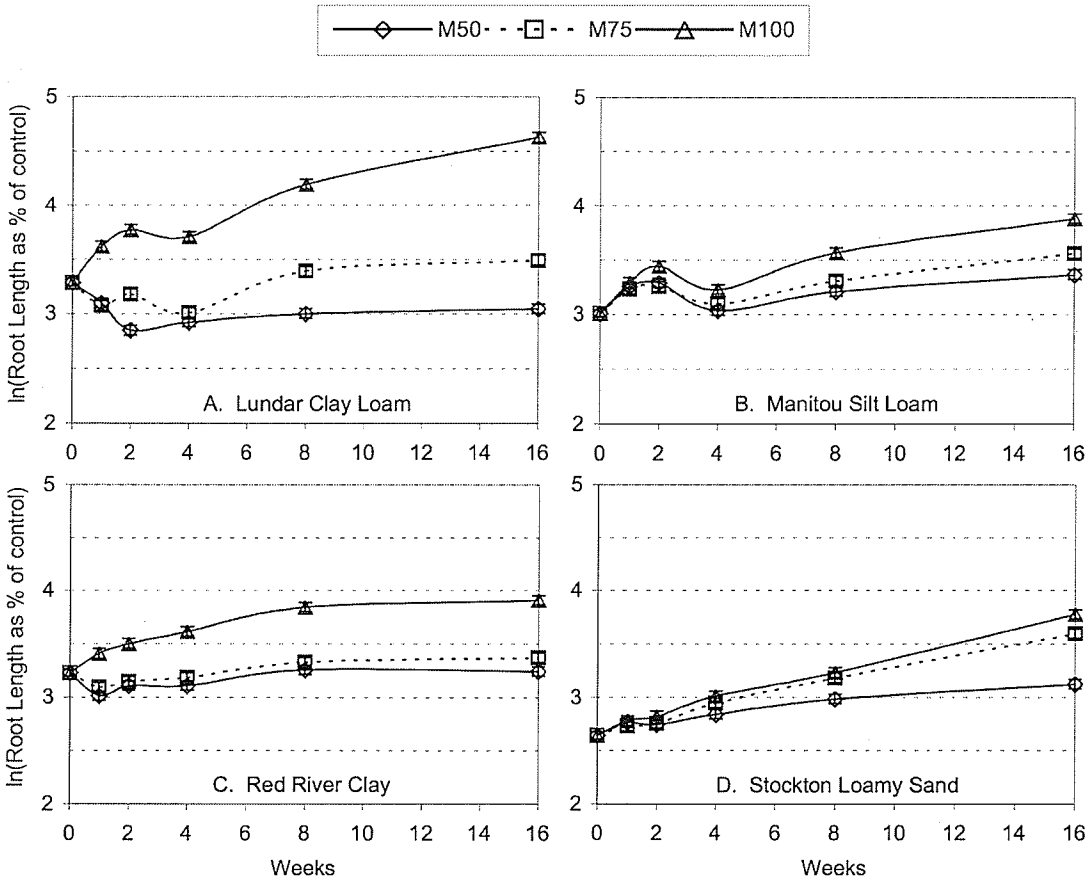

Figure 4.2 Effects of soil properties (A, B, C, D), moisture levels (M50 = 50 % field capacity, M75 = 75 % field capacity, M100 = 100 % field capacity) and duration of incubation (weeks) on oriental mustard root lengths (expressed as the natural logarithm of percent of control) grown in soils treated with Flucarbazone-Sodium at 100 % of its field application dosage on day 0.

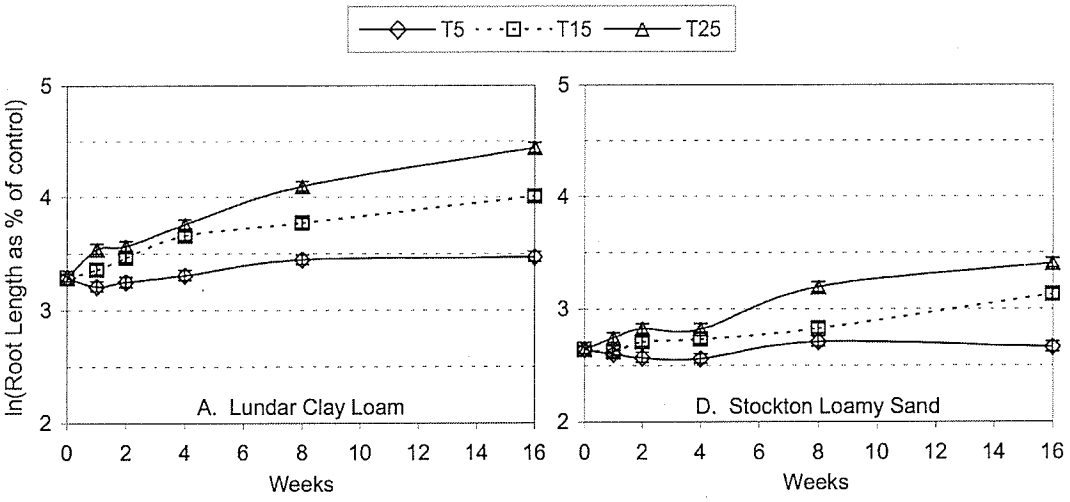

Figure 4.3 Effects of soil properties (A, B), temperature levels (T5 = 5°C, T15 = 15°C, T25 = 25°C) and duration of incubation (weeks) on oriental mustard root lengths (expressed as the natural logarithm of percent of control) grown in soils treated with Imazamox:Imazethapyr (1:1) at 100 % of its field application dosage on day 0.

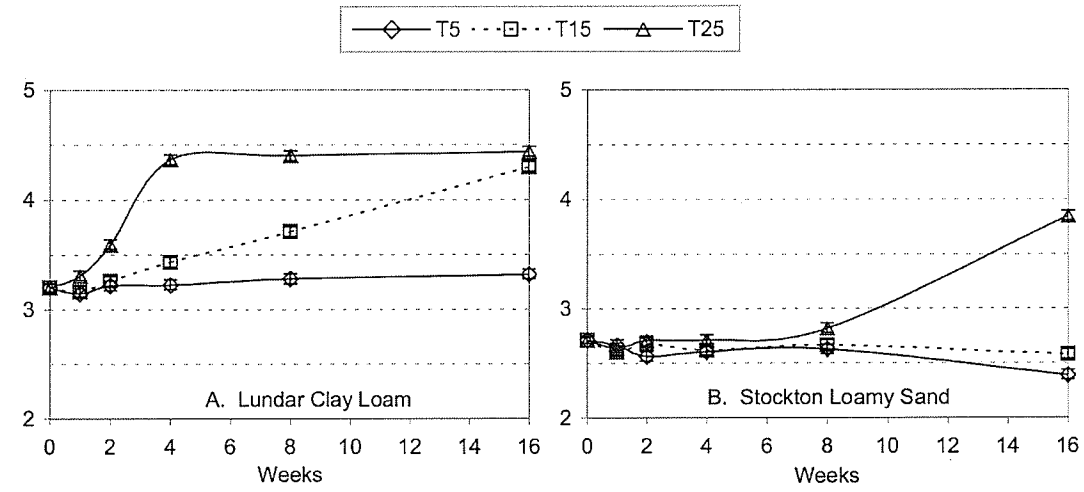

Figure 4.4 Effects of soil properties (A, B), temperature levels (T5 = 5°C, T15 = 15°C, T25 = 25°C) and duration of incubation (weeks) on oriental mustard root lengths (expressed as the natural logarithm of percent of control) grown in soils treated with Flucarbazone-Sodium at 100 % of its field application dosage on day 0.

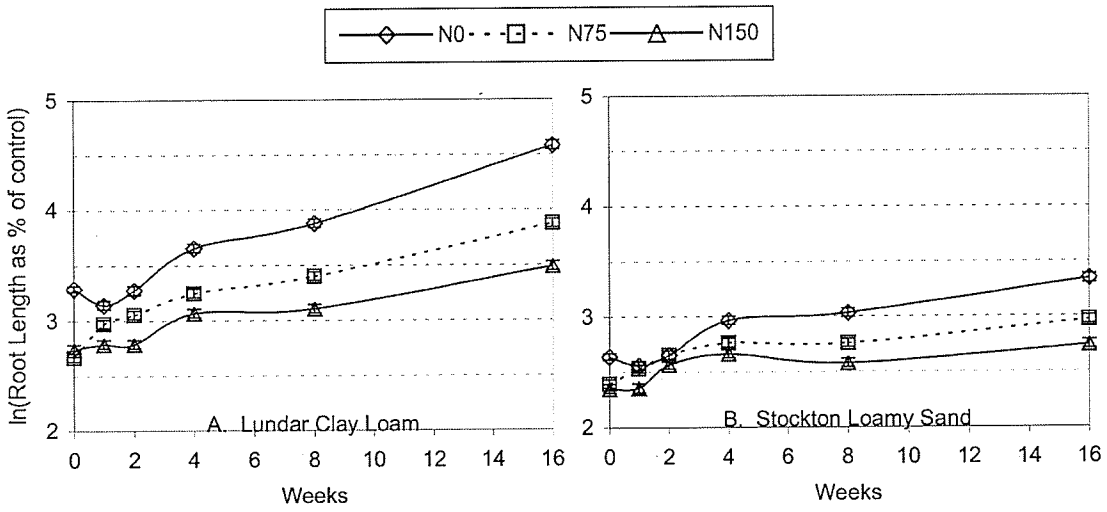

Figure 4.5 Effects of soil properties (A, B), nitrogen levels (N0 = 0 kg N ha^{-1}, N75 = 75 kg N ha^{-1}, N150 = 150 kg N ha^{-1}) and duration of incubation (weeks) on oriental mustard root lengths (expressed as the natural logarithm of percent of control) grown in soils treated with Imazamox:Imazethapyr (1:1) at 100 % of its field application dosage on day 0.

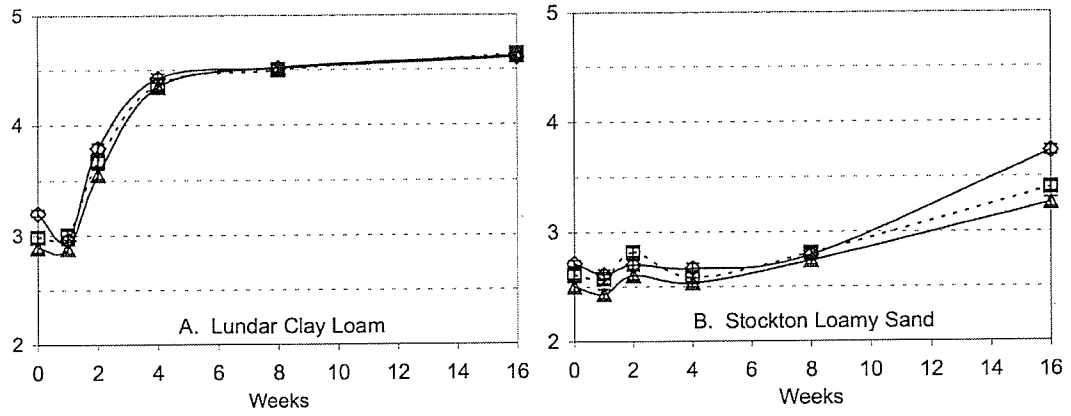

Figure 4.6 Effects of soil properties (A, B), nitrogen levels (N0 = 0 kg N ha^{-1}, N75 = 75 kg N ha^{-1}, N150 = 150 kg N ha^{-1}) and duration of incubation (weeks) on oriental mustard root lengths (expressed as the natural logarithm of percent of control) grown in soils treated with Flucarbazone-Sodium at 100 % of its field application dosage on day 0.

Figures 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6 depict the change in oriental mustard root length measured over incubation time. This is an observation of the change in bioavailability of imazamox:imazethapyr (1:1) and flucarbazone-sodium over time, which is an integrated measure of both herbicide degradation and sorption. For example, the herbicides may continue to persist in the soil, but may not be bioavailable to the oriental mustard due to herbicide sorption by soil constituents. Figures 4.7, 4.8 and 4.9 are the calculated bioavailable concentrations of imazamox:imazethapyr (1:1) and flucarbazone-sodium remaining in the soil after 16 weeks incubation. Again, these do not represent the actual herbicide concentrations remaining in the soil, only that available to and affecting the oriental mustard root growth.

Soil series, soil moisture content (Table 4.1), soil temperature (Table 4.2), nitrogen application rates (Table 4.3) and incubation time were found to all have a significant effect on oriental mustard root length when grown in soils containing either imazamox:imazethapyr (1:1) or flucarbazone-sodium. However, since the interactions of these effects were also significant in all but two cases, individual factors cannot be considered significant, since the effect of one factor is influenced by the effect of another factor. Taking this into account, trends could still be observed regarding the degradation of imazamox:imazethapyr (1:1) and flucarbazone-sodium in the soils studied. Generally, average root lengths (as a percent of control) were longer in flucarbazone-sodium-treated soils as compared to imazamox:imazethapyr (1:1)-treated soils (Table 4.1 and Table 4.3), as expected because the phytotoxicity of imazamox:imazethapyr (1:1) to oriental mustard is greater than that of flucarbazone-sodium (Chapter 2). The exception to this was in the temperature experiment (Table 4.2), for which root lengths grown in flucarbazone-

sodium-treated soil were equal to or less than those grown in imazamox:imazethapyr (1:1)-treated soils. It is difficult to draw individual conclusions from the tables because the root length data for soils (Lundar CL, Manitou SL, Red River C or Stockton LS) are averaged over level and time. Similarly, the root length data given for level are averaged over soil and time; and the root length data given for time are averaged over soil and level. However, an explanation may be found in Figure 4.3 and Figure 4.4. It appears that the degradation of imazamox:imazethapyr (1:1) (i.e. the decreasing bioavailability) is less affected by declining temperature than that of flucarbazone-sodium, which may have had a strong influence on the combined data summaries in Table 4.2.

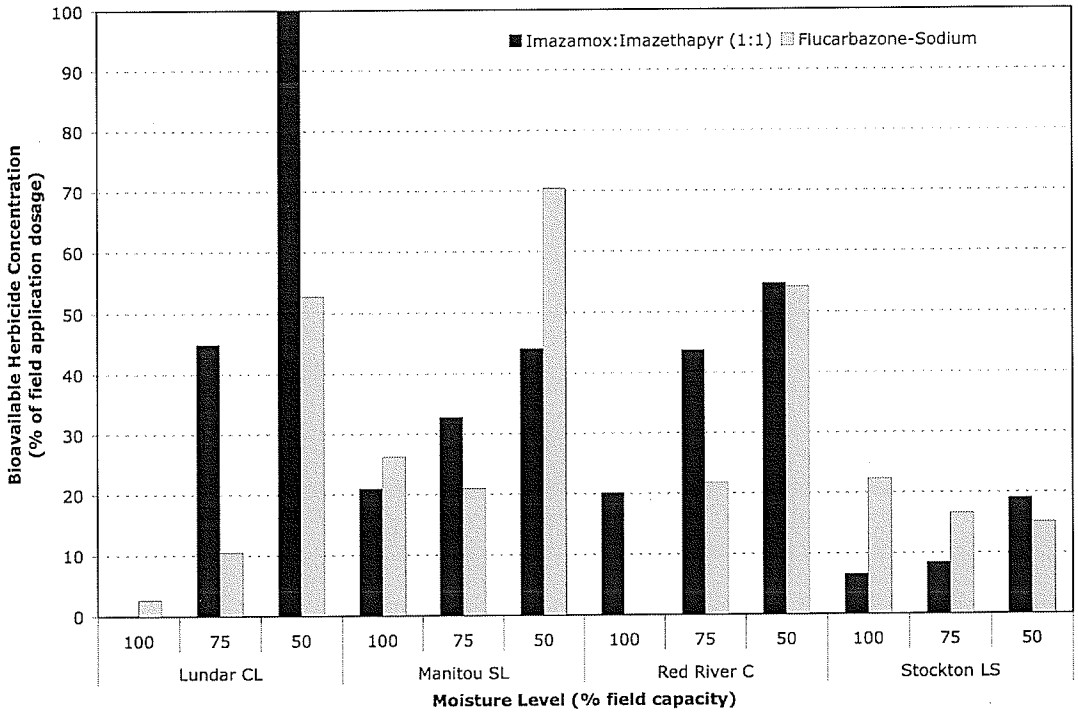

Figure 4.7 Calculated bioavailable concentration of Imazamox:Imazethapyr (1:1) or Flucarbazone-Sodium (% of field application dosage) remaining in the soil (Lundar clay loam, Manitou silt loam, Red River clay, Stockton loamy sand) after 16 weeks of incubation at 50, 75 or 100 % field capacity. 100 % of the field application dosage of either herbicide was applied to the soils on day 0.

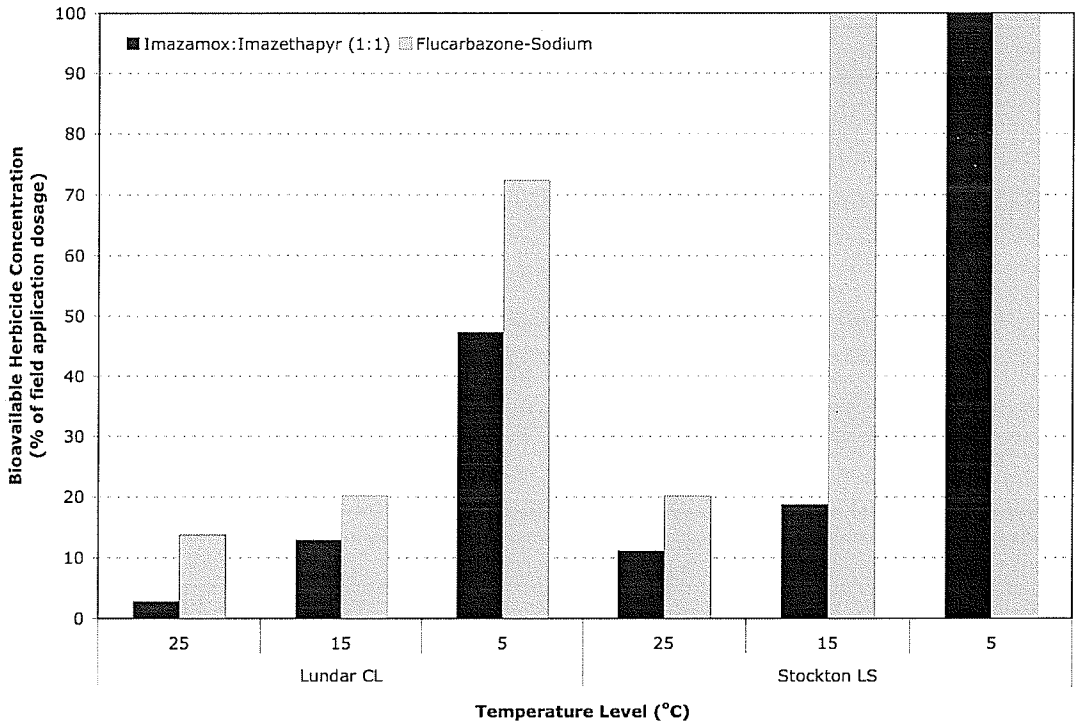

Figure 4.8 Calculated bioavailable concentration of Imazamox:Imazethapyr (1:1) or Flucarbazone-Sodium (% of field application dosage) remaining in the soil (Lundar clay loam, Stockton loamy sand) after 16 weeks of incubation at 25, 15 or 5°C. 100 % of the field application dosage of either herbicide was applied to the soils on day 0.

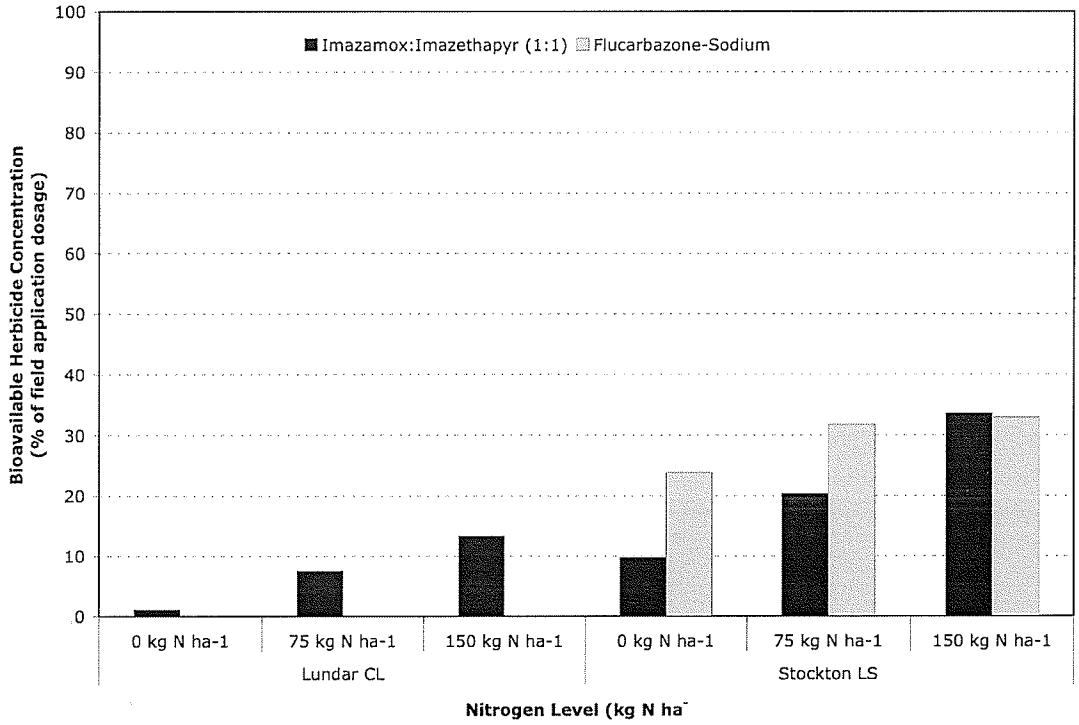

Figure 4.9 Calculated bioavailable concentration of Imazamox:Imazethapyr (1:1) or Flucarbazone-Sodium (% of field application dosage) remaining in the nitrogen-enriched (0, 75 or 150 kg N ha^{-1} ammonium nitrate) soil (Lundar clay loam, Stockton loamy sand) after 16 weeks of incubation. 100 % of the field application dosage of either herbicide was applied to the soils on day 0.

Table 4.1 Soil property, moisture level and incubation time effects on oriental mustard root lengths (expressed as a percent of control) grown in soils treated with Imazamox:Imazethapyr (1:1) or Flucarbazone-Sodium at 100 % of their field application dosages on day 0.

Treatment	Root Length (% of control)	
	Imazamox:Imazethapyr (1:1)	Flucarbazone-Sodium
Soil		
Lundar clay loam	29.38	39.24
Manitou silt loam	26.59	56.83
Red River clay	27.73	39.74
Stockton loamy sand	19.22	19.10
Moisture Level (% of field capacity)		
50	21.30	26.30
75	23.82	40.90
100	32.31	43.63
Incubation Time (weeks)		
0	20.95	21.05
1	22.48	29.47
2	23.46	30.96
4	23.16	40.02
8	29.22	43.57
16	35.97	65.77
P value		
Soil	<0.001	<0.001
Level	<0.001	<0.001
Soil x Level	<0.001	<0.001
Time	<0.001	<0.001
Soil x Time	<0.001	<0.001
Level x Time	<0.001	<0.001
Soil x Level x Time	<0.001	<0.001

Table 4.2 Soil property, temperature level and incubation time effects on oriental mustard root lengths (expressed as a percent of control) grown in soils treated with Imazamox:Imazethapyr (1:1) or Flucarbazone-Sodium at 100 % of their field application dosages on day 0.

Treatment	Root Length (% of control)	
	Imazamox:Imazethapyr (1:1)	Flucarbazone-Sodium
Soil		
Lundar clay loam	35.44	34.60
Stockton loamy sand	16.15	15.13
Temperature Level (°C)		
5	19.63	18.45
15	24.19	21.74
25	28.84	29.87
Incubation Time (weeks)		
0	19.39	19.28
1	20.38	18.60
2	21.43	20.20
4	23.14	23.60
8	28.28	25.87
16	33.82	32.43
P value		
Soil	<0.001	<0.001
Level	<0.001	<0.001
Soil x Level	<0.001	<0.001
Time	<0.001	<0.001
Soil x Time	<0.001	<0.001
Level x Time	<0.001	<0.001
Soil x Level x Time	0.0884	<0.001

Table 4.3 Soil property, nitrogen level and incubation time effects on oriental mustard root lengths (expressed as a percent of control) grown in soils treated with Imazamox:Imazethapyr (1:1) or Flucarbazone-Sodium at 100 % of their field application dosages on day 0.

Treatment	Root Length (% of control)	
	Imazamox:Imazethapyr (1:1)	Flucarbazone-Sodium
Soil		
Lundar clay loam	26.51	47.50
Stockton loamy sand	14.84	16.18
Nitrogen Level (kg N ha^{-1})		
0	25.83	29.79
75	18.94	27.97
150	15.96	25.57
Incubation Time (weeks)		
0	14.60	16.74
1	15.26	15.54
2	16.98	24.32
4	21.37	32.75
8	22.78	38.34
16	33.07	57.13
P value		
Soil	<0.001	<0.001
Level	<0.001	<0.001
Soil x Level	<0.001	0.10
Time	<0.001	<0.001
Soil x Time	<0.001	<0.001
Level x Time	<0.001	<0.001
Soil x Level x Time	0.04	<0.001

4.4.2.1 Soil Moisture. Root lengths observed in soils containing imazamox: imazethapyr (1:1) were numerically shorter than in soils containing flucarbazone-sodium (Figure 4.1 and 4.2, Table 4.1). This was expected, since oriental mustard is more sensitive to imazamox: imazethapyr (1:1) than to flucarbazone-sodium as was observed in Chapters 2 and 3. In addition, imazamox:imazethapyr (1:1) has a greater persistence and hence longer bioactivity, because the half-lives reported for flucarbazone-sodium (17 days) are much less than that of imazamox (20-30 days) and imazethapyr (60-90 days) (Vencill, 2002a; Vencill, 2002b; Vencill, 2002c).

The rate of herbicide degradation in a soil is influenced by the activity of the microbial biomass, which is, in turn, controlled by environmental factors and availability of the substrate (Anderson, 1984). Thus, environmental extremes, such as dry soil moisture conditions (e.g. 50 % field capacity), can severely diminish soil microbial activity, increasing herbicide persistence and bioavailability, as we observed for drier soils treated with either imazamox:imazethapyr (1:1) (Figure 4.1) or flucarbazone-sodium (Figure 4.2). For imazamox:imazethapyr (1:1)-treated soils, the root length curves for the 50 and 75 % field capacity levels are more similar, except in the Stockton LS soil for which the root length curves for the 75 and 100 % field capacity levels follow the same pattern (Figure 4.1). In the flucarbazone-sodium-treated soils, the root length curves for the 75 and 100 % field capacity levels are very similar (Figure 4.2). This suggests that the degradation rate of flucarbazone-sodium in Manitoba soils is relatively constant under field capacities ranging from 75 to 100 %, but that the rate of degradation is strongly reduced under drier soil conditions. This also indicates that in wetter soils, flucarbazone-

sodium bioavailability is less affected by decreasing moisture than imazamox: imazethapyr (1:1) bioavailability.

Soil moisture contents have a smaller effect on herbicide sorption than on herbicide degradation rates. For example, Koskinen et al. (2002) found no effect of moisture on the sorption of flucarbazone-sodium, thus the effect of moisture is on flucarbazone-sodium degradation. This was observed by Eliason et al. (2004), who found that flucarbazone-sodium was more persistent in drier soils (half-lives of 11 days in soil at 85 % field capacity and 25 days in soil at 50 % field capacity). Similarly, Aichele and Penner (2005) found that difference between moisture levels did not significantly affect adsorption and desorption of imazamox or imazethapyr. A number of studies have found that higher soil moisture resulted in enhanced degradation of imazethapyr (Flint and Witt, 1997; Goetz et al., 1990).

For both imazamox:imazethapyr (1:1) and flucarbazone-sodium, oriental mustard root length increased with incubation time (Figure 4.1 and 4.2, Table 4.1) indicating progressively lesser herbicide bioavailability over time. Several studies have reported on increased sorption over time, which affects herbicide degradation rates and hence overall herbicide bioavailability. For example, Koskinen et al. (2002) found that aging (i.e. incubation) significantly increased sorption of flucarbazone-sodium. The greatest increase in sorption was observed during the first two weeks of incubation, where Kd values increased by a factor of four for a clay loam soil, and by a factor of 6.8 for a loamy sand. This increase in sorption could have resulted in the large increase in root lengths observed in the first four weeks of the flucarbazone-sodium-treated Lundar CL, Manitou SL and Red River C soil (Figure 4.2). The Stockton LS did not show a large increase in

root length in the flucarbazone-sodium treatment until the eight to 16 week period, likely due to its low sorptive capacities (low organic matter and low clay content (Table 2.1, Table 2.6)) and hence prolonged herbicide bioavailability. Increases in imazamox and imazethapyr sorption over time have also been observed, however increases in imazamox sorption were only seen at lower soil pH (5.4) (Bresnahan et al., 2002; Bresnahan et al., 2000). The increases in sorption reported by Bresnahan et al. (2002; 2000) for imazamox and imazethapyr occurred more slowly and were not as great as those observed by Koskinen et al. (2002) for flucarbazone-sodium. This could also partially explain why the average increase in root length from week 0 to week 16 was much greater for flucarbazone-sodium than for imazamox:imazethapyr (1:1) (Table 4.1), and why the increase in root length over time occurred much more slowly for imazamox:imazethapyr (1:1) than for flucarbazone-sodium (Figure 4.1 and 4.2).

It appears that the effect of decreasing moisture was greater in the Lundar CL as compared to the other three soils (Figure 4.1, Figure 4.7). For example, after 16 weeks of incubation at 100 % field capacity, a very small portion of bioavailable imazamox:imazethapyr (1:1) remained in the Lundar CL, relative to the amounts of bioavailable imazamox:imazethapyr (1:1) remaining in Manitou SL and Red River C soils (Figure 4.3). In contrast, at the 75 and 50 % field capacity levels, the amount of bioavailable imazamox:imazethapyr (1:1) was similar or larger in the Lundar CL than the Manitou SL or Red River C soils (Figure 4.3). Thus, at the 100 % field capacity, the bioavailability of imazamox:imazethapyr (1:1) over time decreased more rapidly in the Lundar CL as compared to the Manitou SL and Red River C (Figure 4.1, Figure 4.7). For Manitou SL, this may be a function of the sorption and desorption processes in this low

pH soil (Table 2.1). Imazamox and imazethapyr have found to sorb more readily to soils with lower pH (<6) as compared to those with higher pH (>6), however more is desorbed at the lower pH levels even after incubation (Ahmad et al., 2001; Aichele and Penner, 2005; Bresnahan et al., 2002; Bresnahan et al., 2000; Loux et al., 1989; Oliveira et al., 1999). Thus, although sorption may have decreased bioavailability in the Manitou SL initially, when soils were brought up to 100 % field capacity to complete the bioassay, desorption may have resulted in increased bioavailability (Figure 4.1). This desorption would not have occurred in the higher pH Lundar CL and Red River C (Table 2.1). In addition, Aichele and Penner (2005) also found that dissipation was faster at pH 7 than at pH 5 for both imazamox and imazethapyr. With respect to the Red River C, which had a pH of 7.4, the greater bioavailability of imazamox:imazethapyr (1:1) after incubation as compared to Lundar CL may have been a result of the low microbial activity observed in the FDA experiment (Section 4.4.1), possibly resulting in slower degradation rates and hence greater herbicide persistence.

For flucarbazone-sodium, root lengths, averaged over time and moisture level, decreased in the order Manitou SL > Lundar CL = Red River C > Stockton LS (Table 4.2). The Stockton LS was expected to show the least dissipation in bioavailability over time, due to its low sorptive capacity. Koskinen et al. (2002) found that with no incubation, average Kd values of flucarbazone-sodium in a clay loam soil (similar to Lundar CL) were 0.65 mL g^{-1}, while in a loamy sand (similar to Stockton LS) 0.11 mL g^{-1} was reported. Koskinen et al. (2002) found that the sorption values increased during incubation to Kd values of 2.59 and 0.75 mL g^{-1} after 12 weeks incubation for the clay loam and loamy sand, respectively (Koskinen et al., 2002). Thus, regardless of the

incubation time, Stockton LS would have continued to display significantly lower sorption than the other soils

4.4.2.2 Soil Temperature. In general, regardless of the herbicide treatment, oriental mustard root lengths were numerically greatest in the warmest soil (25°C) and lowest in the coolest soil (5°C) (Figures 4.3 and 4.4, Table 4.2). From 2 to 8 weeks after flucarbazone-sodium application to soil, the Lundar CL in particular demonstrated a much lesser herbicide bioavailability at 25°C than at 5 and 15°C, suggesting a much faster degradation of flucarbazone-sodium in the warmest soil (Figure 4.4). In the Stockton LS, herbicide bioavailability was constant over time when soil was at 5 and 15°C but decreased for soil at 25°C. Flucarbazone-sodium degradation in the Stockton LS therefore only occurred in the warmest soil (Figure 4.4). In fact, regardless of the herbicide treatment, changes over time in root length at the 5°C incubation level were minimal, indicating both flucarbazone-sodium and imazamox:imazethapyr (1:1) are quite stable at this low temperature (Figure 4.3 and 4.4). This would be expected due to a decrease in microbial activity and thus decreased degradation with decreasing temperature (Anderson, 1984; Flint and Witt, 1997; Jourdan et al., 1998b; Zimdahl et al., 1984). The increased persistence of imazamox:imazethapyr (1:1) with decreasing temperature was also observed by Jourdan et al. (1998b) who found that imazethapyr bioactivity was higher at 10°C than at 27°C and by Flint and Witt (1997) who reported that imazethapyr persisted about two times longer at 15°C than at 30°C. The effect of temperature on sorption is most likely less important. For example, Gaultier et al. (2009) studied the effect of temperature on the sorption and desorption of 2,4-D in wetland sediments and found only a small (i.e. 3 %) significant increase in 2,4-D Kd when the

temperature increased from 5 to 25°C, and no significant differences between temperatures for 2,4-D desorption rates. Jenkins et al. (2000) studied the effect of temperature on the sorption of imazapyr, an imidazolinone, and observed a slower sorption rate for soils at 15 versus 35°C on a soil with pH < 6, however after 48 hours, overall sorption was similar between the two temperatures.

For imazamox:imazethapyr (1:1)-treated soils, all three incubation temperatures demonstrated a similar pattern of herbicide bioavailability over time in both the Lundar CL and Stockton LS, although root lengths were lower for Stockton LS than Lundar CL (Figure 4.3). The greater herbicide bioavailability in the Stockton LS is expected because of its low clay and organic carbon content (Table 2.1), and thus low sorptive capacity (Table 2.6) and microbial biomass. In flucarbazone-sodium-treated soils, the pattern of herbicide bioavailability over time was strongly influenced by temperature, and the response to temperature was different in the Lundar CL and Stockton LS soils (Table 4.2, Figure 4.4). However, in both soils, the degradation of flucarbazone-sodium was relatively rapid under the 25°C incubation, even though the rapid degradation occurred within 4 weeks of herbicide application in the Lundar CL, but not until 8 weeks after herbicide application in the Stockton LS (Figure 4.4). At 16 weeks after herbicide application, the amount of flucarbazone-sodium degraded in the Lundar CL was also relatively large for the 15°C incubation, but there was virtually no degradation of flucarbazone-sodium in the Stockton LS at 15°C (Figure 4.4). Overall, in both soils the amounts of bioavailable herbicides at 16 weeks after application was typically less for imazamox:imazethapyr (1:1) than for flucarbazone-sodium (Figure 4.8).

4.4.2.3 Soil Nitrogen. Root lengths were generally less in the imazamox:imazethapyr (1:1) versus flucarbazone-sodium-treated soils, due to the lesser phytotoxicity of flucarbazone-sodium than imazamox:imazethapyr (1:1) to oriental mustard (Chapter 2). It is possible that this contributed to the fact that the effect of soil nitrogen levels on oriental mustard root length was more pronounced in soils containing imazamox:imazethapyr (1:1) than flucarbazone-sodium (Table 4.3, Figure 4.5, 4.6 and 4.9).

In Lundar CL and Stockton LS soils treated with imazamox:imazethapyr (1:1), herbicide bioavailability increased (i.e. root lengths decreased) with increasing soil nitrogen, suggesting that the addition of nitrogen to soil increases herbicide sensitivity (Figure 4.5). The effect of soil nitrogen on increasing herbicide bioavailability increased with duration of incubation (i.e. the difference between the 0N and 75 and 150N treatments increased over time). For a range of herbicides, including nicosulfuron, another Group 2 ALS inhibitor, Cathcart et al. (2004) measured the impact of nitrogen additions to soil on the efficacy of herbicides to control selected weed species. Cathcart et al. (2004) used low (0.7 mM N) and high (7.7 mM N) nitrogen rates, also achieved through the addition of ammonium nitrate, but unlike the current experiment, the herbicides were sprayed onto plant surfaces rather than soil-applied. For green foxtail and redroot pigweed, the researchers observed that, when plants were grown in soils with greater nitrogen levels, a lesser amount of nicosulfuron was required to induce plant injury. Hence, the observations of Cathcart et al. (2004) agree with the results observed in this imazamox:imazethapyr (1:1) experiment.

Oriental mustard grown in soils treated with flucarbazone-sodium exhibited very small differences in root lengths among soils treated with different nitrogen levels (Figure 4.6). This suggests that the effects of nitrogen levels on herbicide bioavailability is dependent on the herbicide involved and that more research needs to be done in this area. Cathcart et al. (2004) also concluded that the influence of nitrogen on herbicide efficacy is dependent on the type of herbicides involved, as well as the plant species considered. In addition, for soil-residual herbicides, the current study results suggest that the effect of soil type is also important, because after 16 weeks incubation, there was no bioavailable flucarbazone-sodium remaining in the Lundar CL soil at any nitrogen level (Figure 4.9), while the Stockton LS demonstrated herbicide residues under all nitrogen treatments. For Stockton LS, after 16 weeks incubation, soils with nitrogen additions demonstrated smaller root lengths (Figure 4.6) and hence greater amounts of bioavailable herbicide residues (Figure 4.9) than soils without nitrogen additions, again suggesting that herbicide injury may be increased with increased soil nitrogen levels.

4.5 Conclusions

Imazamox:imazethapyr (1:1) and flucarbazone-sodium are Group 2 (ALS-Inhibitor) herbicides whose persistence in soil can lead to crop damage in years following their application. Imazamox:imazethapyr (1:1) and flucarbazone-sodium bioavailability was assessed in four Manitoba soils using the oriental mustard root bioassay procedure applied to soils incubated at varying soil moisture, temperature and nitrogen contents. Generally, imazamox:imazethapyr (1:1) was found to remain more bioavailable over time as compared to flucarbazone-sodium. Degradation of both imazamox:imazethapyr (1:1)

and flucarbazone-sodium was slower at lower soil moistures and temperatures, with both chemicals being particularly stable at 5°C. The addition of nitrogen to soils resulted in decreased oriental mustard root lengths in both Lundar CL and Stockton LS imazamox:imazethapyr (1:1)-treated soils, while the effect was only seen in the Stockton LS flucarbazone-sodium-treated soil. In most cases, even at optimal soil moisture and temperature, some bioavailable herbicide concentration was found to remain after the 16 week incubation period. Thus, persistence into the following growing season would likely occur. In addition, in a field situation where soil moisture and temperature fluctuate throughout the ~16-week growing season, it is unlikely that optimum soil moistures and temperatures would be attained throughout, thus persistence of bioavailable herbicide residues would certainly occur. Producers must therefore be aware of this issue when making recropping decisions, as well as when choosing herbicide rotations.

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5. OVERALL SYNTHESIS

The overall goal of this project was to gain a better understanding of Group 2 herbicide phytotoxicity and dissipation in Manitoba soils. The herbicides studied were imazamox:imazethapyr (1:1) and flucarbazone-sodium – two of the most widely used Group 2's in Manitoba. The first component of this study (Chapter 2) quantified the effect of soil properties and ammonium nitrate application to imazamox:imazethapyr (1:1) and flucarbazone-sodium phytotoxicity using an oriental mustard root bioassay. The second component (Chapter 3) quantified the interaction responses of imazamox:imazethapyr (1:1) and flucarbazone-sodium applied to four southern Manitoba soils using the oriental mustard root bioassay. The third component (Chapter 4) quantified the effects of soil properties, soil moisture, soil temperature and ammonium nitrate application on the dissipation of the bioavailable portions of imazamox: imazethapyr (1:1) and flucarbazone-sodium over time in Manitoba soils.

In the Manitoba soils, imazamox:imazethapyr (1:1) was more phytotoxic to oriental mustard as compared to flucarbazone-sodium, as expected since oriental mustard is more sensitive to imazamox:imazethapyr (1:1) than flucarbazone-sodium. Herbicide sorption to soil decreased herbicide bioavailability to oriental mustard and thus decreased phytotoxicity, as shown by the strong significant negative correlation observed between *Ce* (the amount of herbicide remaining in solution after sorption has reached equilibrium) and oriental mustard root lengths grown in soils containing either imazamox:imazethapyr (1:1) or flucarbazone-sodium. The sorption of imazamox and imazethapyr was found to

have a significant negative correlation with soil pH, which is in agreement with other findings (Ahmad et al., 2001; Bresnahan et al., 2002; Oliveira et al., 2001; Vencill, 2002). Due to the small number of soils studied, there were not significant influences of other soil properties on herbicide sorption. However, Ahmad et al. (2001) reported increased imazethapyr sorption with increasing soil organic matter and clay contents, which is common to many herbicides. Although flucarbazone-sodium sorption could not be studied (due to unavailability of ^{14}C-flucarbazone-sodium), Koskinen et al. (2002) reported higher levels of flucarbazone-sodium sorption in a clay loam (pH 6.2, 30.8 % clay, 3.17 % OC) as compared to a loamy sand (pH 6.7, 5.3 % clay, 0.26 % OC). The effect of soil nitrogen on phytotoxicity was specific to the soil, nitrogen application rate and herbicide application rate. Generally, it appeared that soil nitrogen had a greater effect on imazamox:imazethapyr (1:1) phytotoxicity as compared to flucarbazone-sodium, where increasing nitrogen levels increased herbicide phytotoxicity, especially in the clay loam soil. Increasing the number of soils analyzed for, as well as examining a wider range of nitrogen formulations and herbicides, could help to determine a more definitive relation in future studies. In addition, examining the effect of ammonium nitrate application on imazamox:imazethapyr (1:1) sorption may also help to explain the observed positive correlation between nitrogen level and herbicide phytotoxicity.

The observations regarding herbicide stacking (additive, synergistic or antagonistic effects) varied with soil characteristics and the amounts of herbicide residues present in the soil. Stacking, from an additive response, was observed when flucarbazone-sodium was applied at 100 % of the field application dosage to the Manitou silt loam soil containing imazamox:imazethapyr (1:1) residues, however antagonistic

responses were observed in the remaining three soils. In a field situation, this could occur where a producer planted peas and applied imazamox:imazethapyr (1:1) in year 1, and in year 2, 25 % of the imazamox:imazethapyr (1:1) residues remained in the soil, and the producer planted wheat and applied 100 % of the field application dosage of flucarbazone-sodium. In contrast, when imazamox:imazethapyr (1:1) was applied at 100 % of the field application dosage to soils containing flucarbazone-sodium residues (e.g. year 1 – planted wheat and applied flucarbazone-sodium; year 2 – 25 % flucarbazone-sodium residue carryover, planted peas and applied imazamox:imazethapyr (1:1) at 100 % dosage), stacking, from additive responses, would occur in a wider range of soils: Lundar clay loam, Manitou silt loam and Red River clay. Stacking, from synergistic responses, was also observed when imazamox:imazethapyr (1:1) was applied at lower dosages (< 30 %) to soils containing flucarbazone-sodium residues. This might occur in a 3-year field situation where: year 1 – planted peas and applied imazamox:imazethapyr (1:1); year 2 – planted wheat and applied flucarbazone-sodium; year 3 – < 30 % imazamox:imazethapyr residue carryover from year 1 and 25 % flucarbazone-sodium residue carryover from year 2 synergistically affect the crop growth of the sensitive crop grown in year 3. This would indicate that in order to minimize herbicide stacking, it should be recommended to wait four years between Group 2 herbicide applications to the same field.

These laboratory results generally agree with field studies completed in Saskatchewan soils. Geisel et al. (2008) observed additive responses in three field experiments conducted on clay loam, silt clay and loam soils in central Saskatchewan, where two ALS-inhibiting herbicides (including imazamox:imazethapyr (1:1) and

flucarbazone-sodium) were sequentially applied over the course of two years. Variations in herbicide stacking were observed by field studies completed by Johnson et al. (2005) who applied various Group 2 herbicides in successive years to a eight sites in Saskatchewan. Johnson et al. (2005) observed synergistic reductions in yield at only one site out of eight, resulting from imazamox:imazethapyr (1:1) application in year 1 followed by flucarbazone-sodium or florasulam applications in year 2. They attributed this to the low soil pH, low organic matter content, and low growing season precipitation during the study years, which would have allowed for increased imazamox:imazethapyr (1:1) carryover. It is important to note that variations in herbicide stacking observed in our laboratory study are indicative of what has been found in field studies. Thus, laboratory studies, which can be completed with less quantity of soil and over less time, can be used in lieu of, or complimentary to field studies, although direct extrapolation to field conditions should be done with caution. This laboratory design was to imitate 25 % residue carryover from herbicide applied in year 1, with simultaneously application of the second herbicide at increasing herbicide dosages. This would differ in a field situations where the herbicide applied in the previous year would have been exposed to the complex processes of herbicide aging in soil (e.g. incorporation of herbicide in metal-organic complexes), perhaps leading to changes in the herbicide chemical composition and its effect on plants. In addition, 25 % residue carryover is considered a conservative approach, where 25 % is the level of residue carryover that is expected in a "normal year", while up to 50 % carryover can be expected in a "dry year" (B. Murray, 2009, personal communications). Finally, the bioassays in our laboratory experiments were conducted under conditions of optimal temperature and soil moisture, and thus the

common stresses (e.g. cool soils) that seedlings are exposed to in the spring, would likely have an effect on how herbicide residues would affect seedling growth and crop development in a field situation.

Generally, imazamox:imazethapyr (1:1) was found to remain more bioavailable over time as compared to flucarbazone-sodium in the Manitoba soils studied, likely indicative of the greater sensitivity of oriental mustard to imazamox:imazethapyr (1:1) as compared to flucarbazone-sodium. Differences observed between soils were based on the sorption and desorption capacities and the microbial activity of the soils, where soils low in pH (specifically for imazamox:imazethapyr (1:1)), organic matter, clay content and microbial biomass showed increased bioavailable concentrations remaining after the 16 week incubation period. Dissipation of both imazamox:imazethapyr (1:1) and flucarbazone-sodium was greatest at 100 % field capacity and at 25°C, declining with decreasing soil moisture contents or soil temperatures, because of the lesser herbicide degradation in the drier and cooler soils. The effect of soil moisture on herbicide dissipation was greater for imazamox:imazethapyr (1:1), while temperature had a greater effect on flucarbazone-sodium dissipation. Differences between the nitrogen treatments were minimal in soils containing flucarbazone-sodium, and more pronounced in soils containing imazamox:imazethapyr (1:1), where phytotoxicity increased with increasing soil nitrogen, indicating greater herbicide sensitivity with increasing soil nitrogen contents.

In most cases, even at optimal soil moisture and temperature, some bioavailable herbicide concentration was found to remain after the 16 week incubation period. Thus, persistence into the following growing season would likely occur. In addition, in a field

situation where soil moisture and temperature fluctuate throughout the ~16 week growing season, it is unlikely that optimum soil moistures and temperatures would be attained throughout, thus persistence of bioavailable herbicide residues would certainly occur. Similarly, in our laboratory experiments, we used four soils, with silt loam, clay loam, clay and loamy sand textures, each collected in an agricultural field in Manitoba. It is important for producers to realize that soil characteristics of these soil series could vary between fields because of variations in land management practices. It is also important for producers to realize that the effect of stacking could vary throughout a field because of soil-landscape variations in the soil organic carbon content and soil microbial biomass, which can influence herbicide sorption, bioavailability and degradation (Farenhorst et al., 2008a; Farenhorst et al., 2008b). Producers must therefore be aware of these issues when making recropping decisions, as well as when choosing herbicide rotations. Future studies should consider examining the effect of fluctuations in soil moistures and temperatures on herbicide degradation and phytotoxicity. In addition, measuring phytotoxicity to oriental mustard in field experiments would also be beneficial to gain a better understanding of the effects of field fluctuations in soil properties, soil moisture and soil temperature.

Taking these limitations into account, this research is still valuable in better understanding the processes of imazamox:imazethapyr (1:1) and flucarbazone-sodium residue behaviour in Manitoba soils, and in providing producers general recommendations of how to improve their use of these herbicides within their cropping systems. Results generally agree with the broad recommendations made in the Guide to Crop Protection (Anonymous, 2009a; Anonymous, 2009b; Anonymous, 2009c), however

it may be possible to improve the Guide's recommendations by prioritizing the factors that affect imazamox:imazethapyr (1:1) and flucarbazone-sodium carryover (Table 5.1).

Table 5.1 Prioritization of factors affecting potential for Imazamox:Imazethapyr (1:1) and Flucarbazone-Sodium residue carryover.

Prioritization	Factors Causing Increased Potential for Residue Carryover	
	Imazamox:Imazethapyr (1:1)	Flucarbazone-Sodium
1	Soil Properties	Soil Properties
a	↓ organic carbon	↓ organic carbon
b	↓ clay content	↓ clay content
c	↓ pH	↑ pH[a]
2	↓ soil moisture	↓ soil temperature
3	↓ soil temperature	↓ soil moisture
4	↑ soil nitrogen	↑ soil nitrogen[b]
5	order of application	order of application

[a]Effect of soil pH on Flucarbazone-Sodium residue carryover taken from the Guide to Crop Protection, as the effect of soil pH on sorption was not evaluated in this experiment.
[b]Effect of soil nitrogen content on Flucarbazone-Sodium residue carryover was only observed in a coarse textured soil with low organic carbon content.

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