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Use of Sugar Beet as a Bioindicator Plant for Detection of Flucarbazone and Sulfentrazone Herbicides in Soil

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1. Introduction

Determination of herbicide residues can be challenging due to the very low herbicide concentrations that can persist and remain bioactive in soil. Detection of residual herbicides is of great importance since these miniscule herbicide amounts may cause injury to sensitive rotational crops. Plant bioassays are a valuable alternative to instrumental procedures for determination of herbicides in soil. Instrumental methods such as gas chromatography or high performance liquid chromatography require solvent or solid phase extractions before sample analysis, and these highly efficient extractions enable the determination of total amount of herbicide in soil. In contrast, bioavailable herbicide is determined by bioassay procedures because plant response varies with soil type and generally decreases in soils of high organic matter and clay contents and low soil pH (Thirunarayanan et al. 1985; Renner et al. 1988; Che et al. 1992; Wang & Liu 1999; Wehtje et al. 1987; Grey et al. 1997; Szmigielski et al. 2009). Typically bioassay detection of herbicides that belong to different groups with different modes of action requires use of different plant species and/or measuring different plant parameters. Use of herbicides with different modes of action applied either in rotation or as pre-mixed combinations has become a common practice in farming to combat weed resistance problems. Thus performing more than one bioassay may be necessary for assessment of herbicide residues in soil after field applications of herbicides with different modes of action.

Flucarbazone is used in western Canada for control of certain grass and broadleaf weeds in wheat (*Triticum aestivum* L.) and its recommended application rate is 20 g ai ha⁻¹. Flucarbazone belongs to an acetolactate synthase (ALS) group of herbicide; these herbicides inhibit the biosynthesis of branched amino acids (valine, leucine and isoleucine) and affect primarily root growth of susceptible plants through inhibition of cell division at the root tips. Flucarbazone is a weak acid (pKa = 1.9) and therefore it is present mostly in the anionic form at environmentally relevant pH levels (Senseman 2007). Flucarbazone dissipation rate in soil is fast and the flucarbazone half-life in different soil types has been reported to range from 6 to 110 days (Eliason et al. 2004). However, as is the case with other ALS-inhibiting herbicides (Goetz et al. 1990; Anderson & Humburg 1987; Anderson & Barrett 1985; Loux & Reese 1992; Walker & Brown 1983), flucarbazone may persist in soil particularly under

conditions of low moisture and cool temperature. Residual activity of a herbicide in soil is desirable in providing weed control late in the season; however, if the herbicide persists to the following year, it may damage rotational crops as has been reported for various sensitive crops seeded one year after an ALS-herbicide application including canola (*Brassica napus* L.), flax (*Linum usitatissimum* L.), lentil (*Lens culinaris* Medic), oriental mustard (*Brassica juncea* L.), corn (*Zea mays* L.), and sugar beet (*Beta vulgaris* L.) (Bresnahan et al. 2000; Moyer et al. 1990; Moyer & Esau 1996; Moyer & Hamman 2001).

Sulfentrazone is a soil applied herbicide and is registered in western Canada for control of grass and broadleaf weeds in chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), and flax at application rates of 105 to 140 g ai ha⁻¹. It is a protox herbicide and its mode of action is the inhibition of protoporphyrinogen oxidase that leads to the disruption of lipid cell membranes and consequently causes shoot desiccation after plants emerge from soil and are exposed to light. Sulfentrazone is a weak acid with a pKa of 6.56; therefore, it exists predominantly in ionized form in soils with a pH higher than the pKa (Senseman 2007). Sulfentrazone is relatively persistent in soil, with a half-life reported in the range of 24 to 302 days (FMC Corporation 1999; Martinez et al. 2008; Ohmes et al. 2000). Because of slow sulfentrazone dissipation in some soils especially under conditions of drought and cool weather, a potential risk of carry-over to rotational crops is of concern. Injury to cotton (*Gossypium hirsutum* L.) (Ohmes et al. 2000; Main et al. 2004; Pekarek et al. 2010), sugar beet, and sorghum (*Sorghum bicolor* L.) (FMC Corporation 1999) has been reported one year after sulfentrazone application, and consequently extended recropping intervals are advised for these crops. In western Canada, lentil has exhibited sensitivity to sulfentrazone residues (Johnson E.N. unpublished data) and re-cropping intervals of 36 months are recommended.

This review presents our research on (1) the development of a sugar beet bioassay for detection of flucarbazone and sulfentrazone in soil, (2) the assessment of flucarbazone and sulfentrazone interactions in soil, (3) the evaluation of the N-fertilizer effect on detection of flucarbazone and sulfentrazone, and (4) the investigation of the landscape effect on flucarbazone and sulfentrazone bioactivity and dissipation in Canadian prairie soils.

2. Sugar beet bioassay

Selecting suitable plant species for a bioassay is critical, and the plant parameter measured in a bioassay has to be sensitive and correlate well with herbicide concentration. Typically, ALS-herbicides are detected using root inhibition bioassays, and various susceptible plant species including oriental mustard (Eliason et al. 2004; Szmigielski et al. 2008), corn (Mersi & Foy 1985; Hsiao & Smith 1983), red beet (Jourdan et al. 1998), and sunflower (*Helianthus annuus* L.) (Hernández-Sevillano et al. 2001; Günther et al. 1993) have been used. Prototox-inhibiting herbicides influence mainly shoot development of sensitive plants, and cotton (Main et al. 2004; Grey et al. 2007) and sugar beet (Szmigielski et al. 2009; Blanco & Velini 2005) have been reported as a suitable species for sulfentrazone detection in soil.

We investigated the use of sugar beet (*cv.* Beta 1385) as a bioindicator plant for detection of both flucarbazone and sulfentrazone in one bioassay, by measuring both root and shoot length reduction of sugar beet in response to these two herbicides. This bioassay is performed in 4-oz Whirl-Pak™ plastic bags that are 16 cm long and 6 cm wide. A quantity of 100 g of soil is wetted to 100% field capacity, and then hand mixed in a plastic dish and

transferred to a Whirl-Pak™ bag. The soil in the bag is gently packed to form a rectangular layer approximately 14 cm deep and 1 cm thick (Fig. 1a).

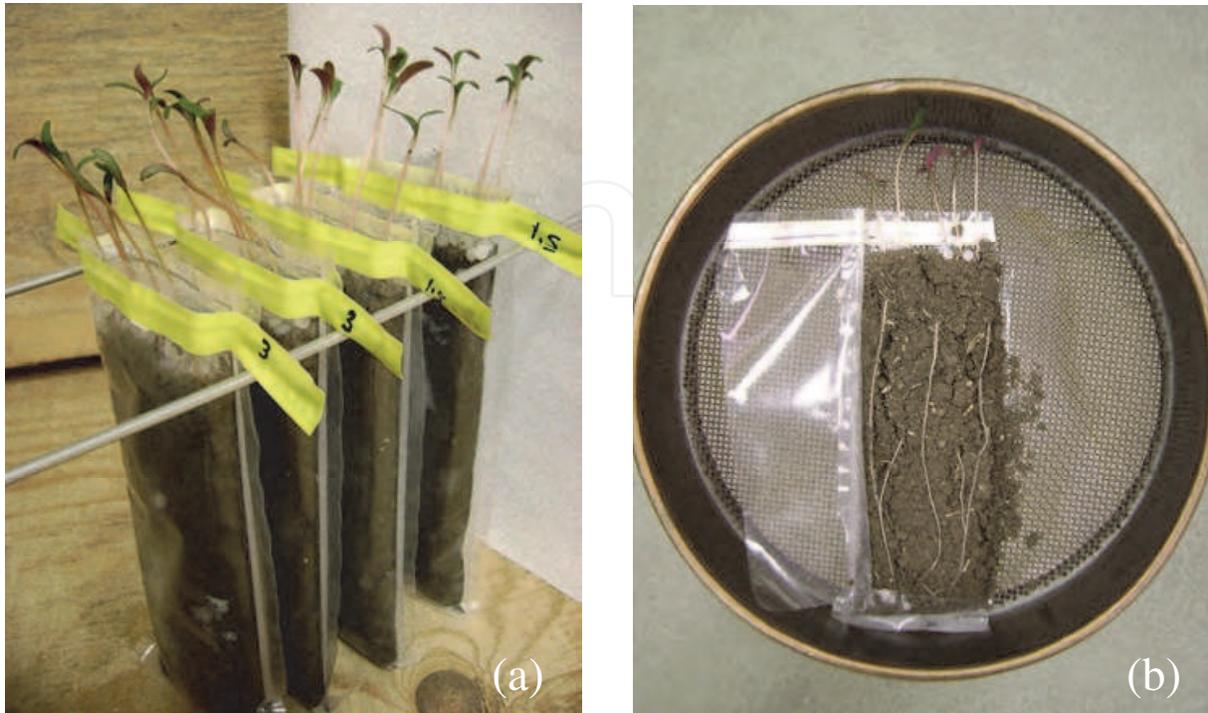


Fig. 1. (a) Sugar beet bioassay performed in WhirlPak™ bags; (b) Opened WhirlPak™ bag on sieve before plant removal from soil with water.



Fig. 2. Response of sugar beet plants to increasing concentration of (a) flucarbazone in the range from 0 to 15 ppb, and (b) sulfentrazone in the range from 0 to 200 ppb in soil.

Six sugar beet seeds are planted and the soil surface is covered with a layer of plastic beads to reduce water evaporation. Plants are then grown in a fluorescent canopy with light intensity of approximately $16 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Szmigielski et al. 2009) and watered to 100% field capacity daily by adding water to the predetermined weight. After a 6-day growth period, length of shoots is measured with a ruler from the soil level to the node where the cotyledons split from the stem. Next, plants are recovered from soil after the Whirl-Pak™ bag is cut open with scissors and placed on a sieve (Fig. 1b); soil is then washed away with water and root length measured with a ruler (Fig. 2a and 2b).

Sugar beet response to flucarbazone in the range from 0 to 15 ppb and to sulfentrazone in the range from 0 to 200 ppb was assessed, and root and shoot inhibition calculated using the formula (Beckie & McKercher 1989):

$$\text{Inhibition (\%)} = (1 - L_t/L_0) \times 100\% \quad (1)$$

where L_t is the root or shoot length in the herbicide-treated soil and L_0 is the root or shoot length in the untreated (control) soil. Dose-response curves can be constructed using a log-logistic regression model (Seefeldt et al. 1995):

$$\text{Inhibition (\%)} = 100\% - (C + [D - C]/[1 + \{x/I_{50}\}^b]) \quad (2)$$

where x is herbicide concentration, $(100 - C)$ is the upper limit of the log-logistic inhibition curve, $(100 - D)$ is the lower limit of the log-logistic inhibition curve, I_{50} is the concentration required for 50% plant growth inhibition, and b is the slope of the curve around the I_{50} value. Measuring both roots and shoots of sugar beet revealed that although flucarbazone primarily inhibits root length it also causes shoot reduction (Fig. 3a), and that while sulfentrazone primarily inhibits shoot length it also affects root development (Fig. 3b).

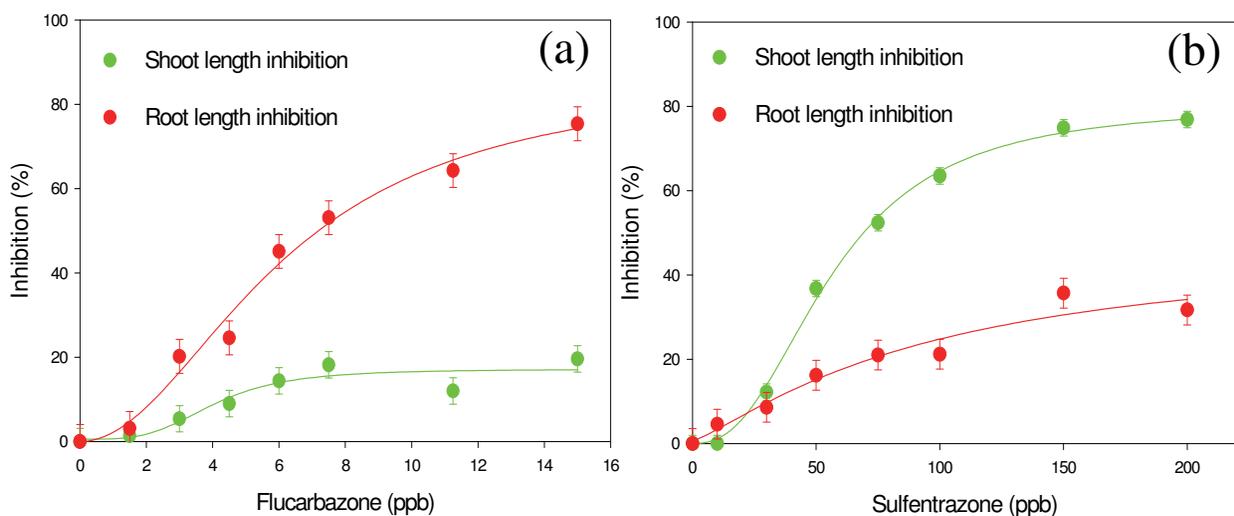


Fig. 3. Shoot and root length inhibition of sugar beet in response to (a) increasing concentration of flucarbazone, and (b) increasing concentration of sulfentrazone in soil.

This sugar beet bioassay is sensitive; concentration of approximately 2 ppb of flucarbazone based on root length measurements and of approximately 20 ppb of sulfentrazone using shoot length measurements was detected. However, bioassay detection limits vary with soil

type and are generally lower in sandy soils of low organic matter content and high pH (Jourdan et al. 1998; Eliason et al. 2004; Szmigielski et al. 2009).

Since flucarbazone and sulfentrazone decrease both root and shoot length of sensitive plants such as sugar beet, sequential or simultaneous applications of these two herbicides could potentially result in herbicide interactions.

3. Flucarbazone and sulfentrazone interactions

Repeated applications of herbicides with the same mode of action have resulted in weeds developing resistance (Vencill et al. 2011; Colborn & Short 1999; Whitcomb 1999). Using herbicides with different mode of action either applied as pre-mixed combinations or applied in rotation reduces problems related to weed resistance and consequently improves weed control. However, combinations of herbicides are generally chosen to improve the spectrum of weed control without prior knowledge of the possible consequences of the interactions between herbicides (Zhang et al. 1995). The outcome of the interactions may be synergistic, antagonistic or additive depending on whether the combined effect on the target plants is greater, less than, or equal to the summed effect of the herbicides applied alone (Colby 1967; Nash 1981). A synergistic interaction occurs when the activity of two herbicides is more phytotoxic than either herbicide applied singly. A synergistic effect is beneficial in that it provides more effective weed control at lower herbicide concentrations; however it may also cause injury to sensitive rotational crops if the synergism of the two residual herbicides is not known (Zhang et al. 1995). In an additive interaction, also called "herbicide stacking" (Johnson et al. 2005), the injury observed in the target plants is the sum activity of the combined herbicides. With an antagonistic interaction, the efficacy of the combined herbicides is reduced and consequently results in decreased weed control but can also help to avoid unwanted crop injury (Zhang et al. 1995).

To examine interactions between soil-incorporated flucarbazone and sulfentrazone, we evaluated the combined effect of these two herbicides on sugar beet root and shoot inhibition. Root length inhibition was assessed in soil that was spiked with mixtures consisting of flucarbazone in the range from 0 to 15 ppb with sulfentrazone added at 50 ppb level, while shoot length inhibition was evaluated in soil that was amended with mixtures consisting of sulfentrazone in the range from 0 to 200 ppb with flucarbazone added at 6 ppb level. The expected inhibition was calculated using Colby's formula (Colby 1967):

$$E = X + Y - XY/100 \quad (3)$$

where X is the plant growth inhibition (%) due to compound A and Y is the plant growth inhibition (%) due to compound B; comparing expected inhibition to the observed inhibition allows the nature of interactions to be revealed. The combined effect of flucarbazone and sulfentrazone was additive: the observed and expected root length inhibition of sugar beet in response to flucarbazone in combination with sulfentrazone were similar (Fig. 4a), as were the observed and the expected shoot length inhibition due to sulfentrazone in combination with flucarbazone (Fig. 4b). I_{50} values for observed and expected responses were not different at 0.05 level based on the asymptotic z-test. The additive effect of flucarbazone and sulfentrazone will help in weed control but may also increase risk of injury to rotational crops that are sensitive to both these herbicides.

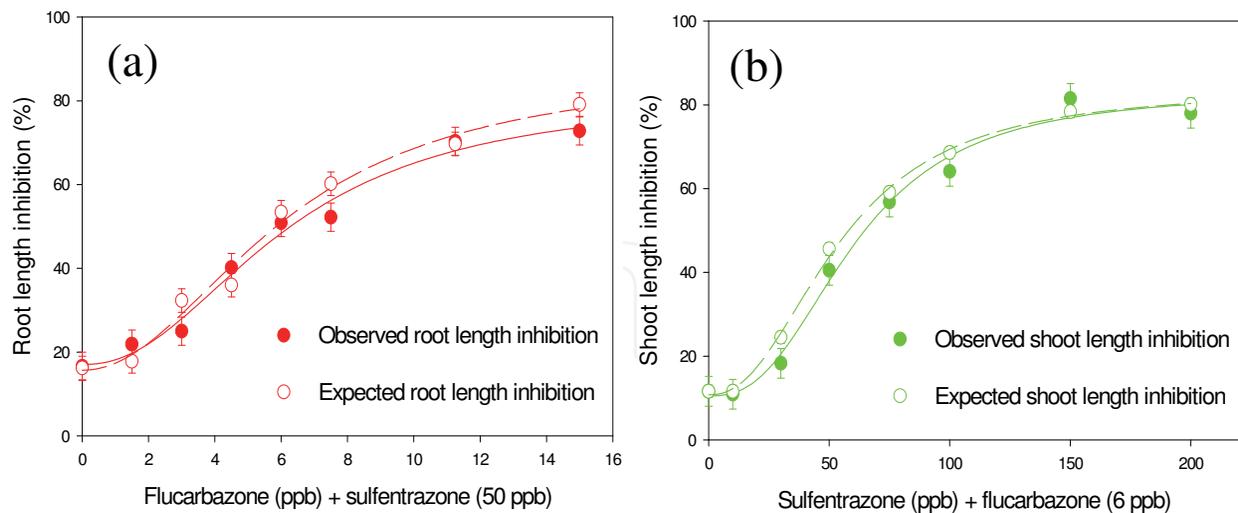


Fig. 4. (a) Root length inhibition of sugar beet in response to increasing concentration of flucarbazone in combination with 50 ppb sulfentrazone, and (b) shoot length inhibition of sugar beet in response to increasing concentration of sulfentrazone in combination with 6 ppb flucarbazone.

4. Effect of ammonium containing fertilizer on sugar beet bioassay

Typically plant response that is measured in a bioassay is not specific to one source. The lack of specificity may be desirable in that the presence of residues of all herbicides that detrimentally affect the same plant parameter are detected. However, other soil applied chemicals apart from herbicides may also alter the parameter measured in a bioassay and may change the outcome of the bioassay. We have reported that the detection of ALS-inhibiting herbicides in soil using a mustard root bioassay is influenced by N-fertilizer as mustard root length is shortened in response to ammonium ions (Szmigielski et al. 2011). Ammonium toxicity to plants is common and a change in root/shoot ratio is one of the symptoms of NH_4^+ toxicity (Britto & Kronzucker 2002).

To assess the effect of N-fertilizer on sugar beet roots and shoots, and consequently on flucarbazone and sulfentrazone detection in soil, ammonium nitrate was added to soil in the range from 0 to 200 ppm N, and root and shoot length was measured. Ammonium nitrate significantly reduced root length of sugar beet but the shoot length inhibition due to ammonium nitrate was very small and was less than 20% at the highest ammonium nitrate concentration tested (Fig. 5).

The combined response of sugar beet roots to flucarbazone and ammonium nitrate was examined by growing sugar beet plants in soil that was spiked with flucarbazone in the range of 0 to 15 ppb and mixed with ammonium nitrate added at 50 ppm N. The expected response due to flucarbazone in combination with ammonium nitrate was calculated using equation [3]. Since the expected root length inhibition was the same as the observed (Fig. 6), the combined effect of flucarbazone and N-fertilizer on sugar beet root length is additive.

Thus, root length reduction of sugar beet that is measured in a soil that received a recent application of ammonium containing or ammonium producing fertilizer may be

misinterpreted as reduction due to herbicide residues and may yield false positive results. Because N-fertilizer interferes with the sugar beet root length bioassay, preferably soil sampling for the detection of residual herbicides should be completed preplant and before N-fertilizer field application, or at the end of the growing season.

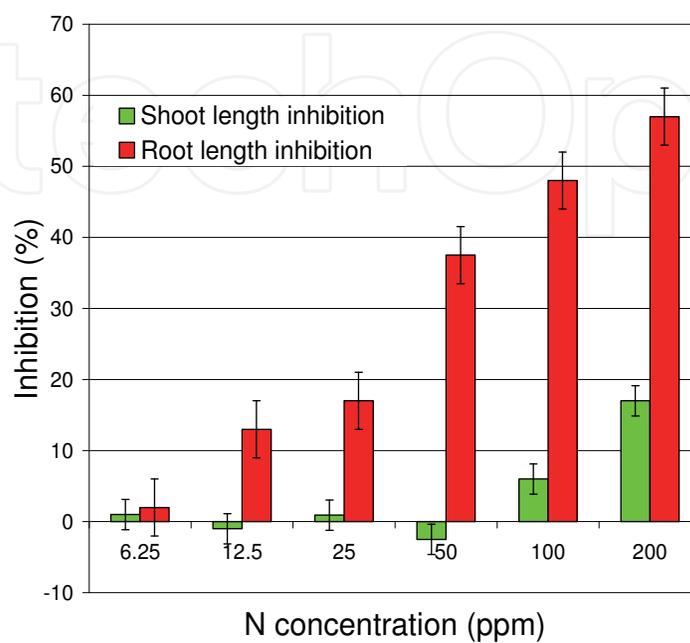


Fig. 5. Effect of increasing ammonium nitrate concentration in soil on shoot and root inhibition of sugar beet plants.

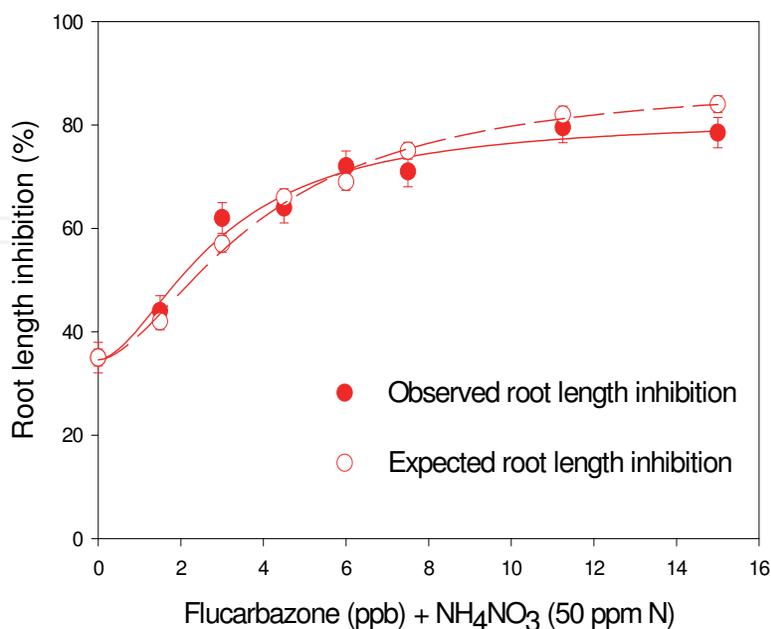


Fig. 6. Root length inhibition of sugar beet in response to increasing concentration of flucarbazone in combination with 50 ppm N added as ammonium nitrate.

5. Effect of landscape position on phytotoxicity and dissipation of flucarbazone and sulfentrazone

Farm fields with irregular rolling topography of low hills and shallow depressions are typical on the Canadian prairies (Fig. 7). Low-slope soils from depressions in the landscape typically have higher organic matter and clay contents and lower pH than up-slope soils from elevated parts of the terrain (Schoenau et al. 2005; Moyer et al. 2010). Furthermore, low-slope areas in the field generally have higher moisture content as a result of water accumulating in the depressions, while the up-slope areas are drier due to water runoff.



Fig. 7. Undulating landscape comprised of knolls and depressions in southwestern Saskatchewan (source: Geological Survey Canada).

Phytotoxicity of ALS- and protox-inhibiting herbicides is soil dependent, and the effect of organic matter, clay and soil pH on adsorption and bioavailability of these herbicides is well documented (Thirunarayanan et al. 1985; Renner et al. 1988; Che et al. 1992; Wang & Liu 1999; Wehtje et al. 1987; Grey et al. 1997; Szmigielski et al. 2009). Typically organic matter and clay decrease the concentration of bioavailable herbicide through adsorption of herbicide molecules to the reactive functional groups and colloidal surfaces. At alkaline soil pH, adsorption of weak acidic herbicides tends to decrease due to increased herbicide solubility in soil solution and due to repulsion of anionic herbicide molecules from negatively charged soil particles.

Dissipation of ALS- and protox-inhibiting herbicides in soil is governed by microbial and chemical processes. Microbial degradation is the primary mechanism as dissipation has been shown to be faster in non-sterile soil than in autoclaved soil (Joshi et al. 1985; Ohmes et al. 2000; Brown 1990). The dissipation rate of ALS- and protox-inhibiting herbicides varies with soil type and environmental conditions. Generally high organic matter content, high clay content and low soil pH decrease the dissipation rate by reducing the amount of herbicide available in soil solution for decomposition (Eliason et al. 2004; Goetz et al. 1990; Beckie & McKercher 1989; Ohmes et al. 2000; Grey et al. 2007; Main et al. 2004). Microbial and chemical decomposition both depend on soil water and temperature with faster dissipation occurring in moist and warm soils (Beckie & McKercher 1989; Joshi et al. 1985;

Walker & Brown 1983; Brown, 1990; Thirunarayanan et al. 1985). In flooded (saturated) soils decomposition may be reduced due to anaerobic conditions.

To examine the effect of landscape position on phytotoxicity and dissipation of flucarbazone and sulfentrazone, we used two soils that were collected from a farm field with varying topography in southern Saskatchewan, Canada. Soil from an up-slope position contained 0.9% organic carbon, 31% clay and had pH 7.9, while soil from a low-slope position contained 1.6% organic carbon, 51% clay and had pH 7.2. Flucarbazone phytotoxicity was assessed in the range from 0 to 15 ppb by measuring root length inhibition while sulfentrazone phytotoxicity was determined in the range from 0 to 200 ppb by measuring shoot length inhibition of sugar beet. Phytotoxicity of flucarbazone (Figure 8a) and of sulfentrazone (Figure 8b) was higher in the up-slope soil than in the low-slope soil. The I_{50} values determined from the dose-response curves were 3.5 and 5.7 ppb for flucarbazone, and 34.3 and 56.5 ppb for sulfentrazone in the up-slope and low-slope soil, respectively, and were different at 0.05 level of significance. Thus landscape position in a field has a considerable effect on bioavailability of flucarbazone and sulfentrazone, and different herbicide application rates may be required in fields of variable topography to achieve uniform weed control.

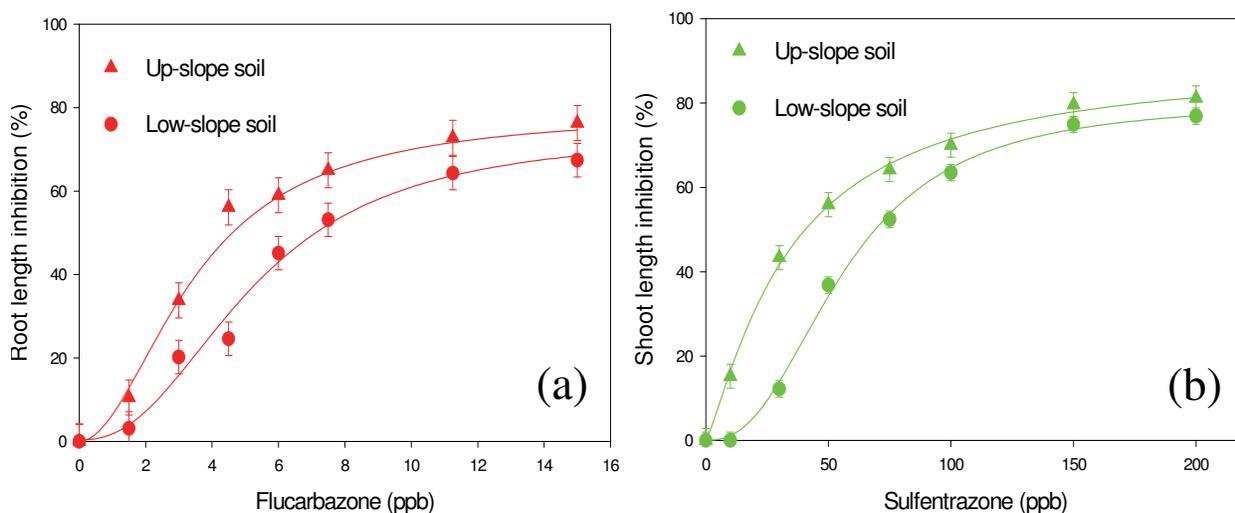


Fig. 8. Dose-response curves for (a) flucarbazone determined by root length, and (b) sulfentrazone determined by shoot length of sugar beet in soil from two landscape positions.

Flucarbazone and sulfentrazone dissipation in the two soils was examined under laboratory conditions of 25 C and moisture content of 85% field capacity. Soils were spiked with 15 ppb of flucarbazone and separately with 200 ppb of sulfentrazone, and at each sampling time the residual flucarbazone and sulfentrazone was determined using the sugar beet bioassay. Flucarbazone and sulfentrazone dissipation followed the bi-exponential decay model described in detail by Hill & Schaalje (1985):

$$C = a e^{-bt} + c e^{-dt} \quad (4)$$

where C is herbicide concentration remaining in soil after time t. In the bi-exponential decay model the dissipation rate is not constant and is fast initially and slow afterward,

while in the first order decay model (when $b = d$ in equation [4]) the dissipation rate does not change with time. Flucarbazone and sulfentrazone dissipation was more rapid in the up-slope soil than in the low-slope soil (Fig. 9a and 9b); flucarbazone half-life was 5 and 8 days, and sulfentrazone half-life was 21 and 90 days in the up-slope and the low-slope soil, respectively. Thus landscape positions in the field influence persistence of flucarbazone and sulfentrazone, and consequently may affect the potential for herbicide carry-over to the next growing season. However, because damage to sensitive rotational crops occurs when a herbicide is available to plants at harmful concentrations one year after application, risk of carry-over injury is controlled by the combined effect of herbicide dissipation and herbicide phytotoxicity, both of which are soil dependent; also the rotational crop must be susceptible to the residual herbicide concentration at the time of planting (Hartzler et al. 1989). Although flucarbazone and sulfentrazone persist longer in soil from depressions in the field, herbicide bioavailability is reduced in this soil, and thus residual flucarbazone or sulfentrazone may not pose a risk of injury to sensitive crops in low-slope areas. Predicting carry-over injury due to flucarbazone and sulfentrazone in farm fields with varying topography is a complex task and all factors that affect herbicide persistence and bioavailability have to be considered before choosing a rotational crop to grow.

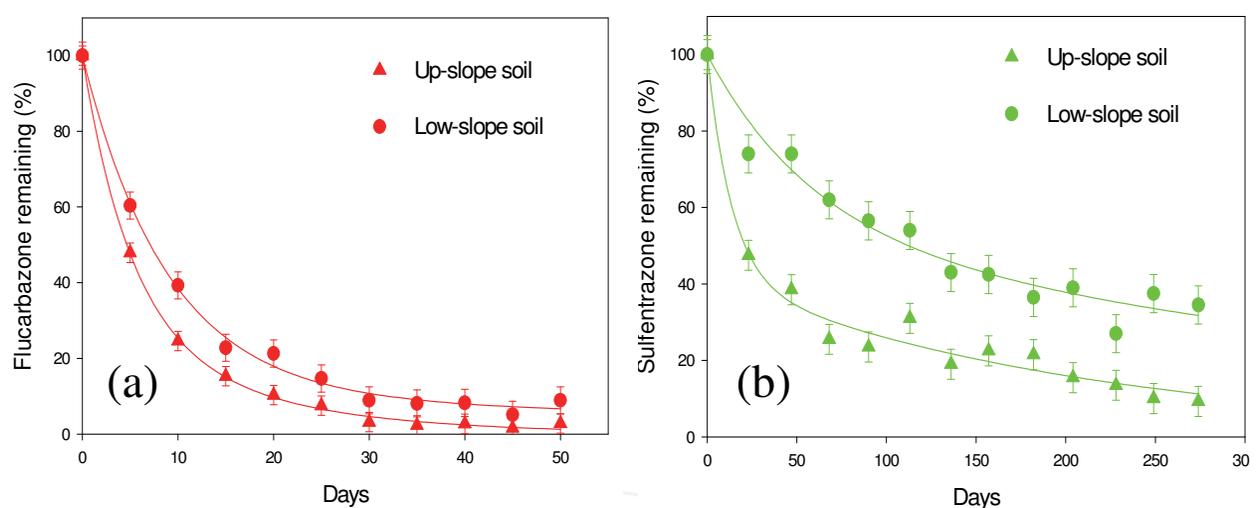


Fig. 9. Dissipation under laboratory conditions of (a) flucarbazone determined by root length, and (b) sulfentrazone determined by shoot length of sugar beet in soil from two landscape positions.

6. Practical considerations

Because sugar beet plants respond both to flucarbazone and sulfentrazone, a sugar beet bioassay allows for detection of these two herbicides in soil by evaluating both root and shoot inhibition. Growing sugar beet plants in Whirl-Pak™ bags is simple and provides a convenient method for assessing shoot and root length. Shoots are measured above the soil level and do not need to be harvested; this helps particularly with measuring shoots that are short and brittle at phytotoxic sulfentrazone concentrations. Roots are recovered from soil with water and consequently roots do not get broken or damaged before being measured.

Furthermore, as the bioassay is completed before roots grow to the bottom of the bag, root development in Whirl-Pak™ bags is not obstructed.

7. Conclusions

Using the sugar beet bioassay we determined: (1) that while flucarbazone primarily inhibits root length it also causes shoot reduction and while sulfentrazone primarily inhibits shoot length it also affects root development, (2) that the combined effect of soil-incorporated flucarbazone and sulfentrazone on root and shoot length inhibition of sugar beet is additive, (3) that N-fertilizer reduces root length of sugar beet but has little effect on shoot length and therefore the presence of freshly applied N-fertilizer may yield false positive results for flucarbazone residues, and (4) that flucarbazone and sulfentrazone phytotoxicity is higher and dissipation rate is faster in soils from up-slope than low-slope landscape positions under identical moisture and temperature conditions.

8. Acknowledgements

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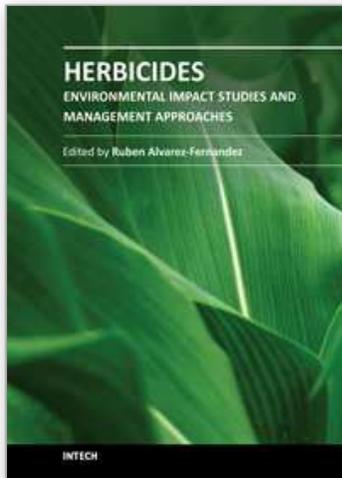
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Weeds severely affect crop quality and yield. Therefore, successful farming relies on their control by coordinated management approaches. Among these, chemical herbicides are of key importance. Their development and commercialization began in the 1940's and they allowed for a qualitative increase in crop yield and quality when it was most needed. This book blends review chapters with scientific studies, creating an overview of some the current trends in the field of herbicides. Included are environmental studies on their toxicity and impact on natural populations, methods to reduce herbicide inputs and therefore overall non-target toxicity, and the use of bioherbicides as natural alternatives.

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