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## Interactions of Soil Residual ALS Inhibiting Herbicides

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### Abstract

ALS inhibiting herbicides exhibit high bioactivity at low concentrations and may persist in the soil. To examine possible interactions between two different residues present together in the soil, field and lab tests were performed in three contrasting Saskatchewan soils. Field plots using Roundup Ready canola were used to assess residual effects of combinations of ALS inhibiting herbicides applied to peas and wheat in the previous two years. A root length inhibition bioassay based on oriental mustard was used to test for residual herbicide phytotoxicity in samples of soil from field and lab-spiked soils from the three study sites. The field plots were sprayed initially with imazamox/imazethapyr, and followed by imazamethabenz, flucarbazone-sodium, sulfosulfuron, or florasulam in the second growing season. Soil samples were taken from the plots after the second growing season for the bioassay test. To determine the interactions (antagonistic, additive, or synergistic) between the herbicides investigated, Colby's equation was applied to the bioassay results. In field samples, the results to date have indicated additive and potential synergistic interactions for the same herbicide combinations in different soils.

### Introduction

The group of herbicides that inhibit acetolactate synthase (ALS) enzyme has become increasingly popular in Western Canadian production agriculture. Imazamox/imazethapyr (Odyssey<sup>®</sup>), a common herbicide used in Western Canada for peas, along with the cereal herbicides imazamethabenz (Assert<sup>®</sup>), flucarbazone-sodium (Everest<sup>®</sup>), sulfosulfuron (Sundance<sup>®</sup>), and florasulam (Frontline<sup>®</sup>) all potentially have soil residual properties. These ALS inhibiting herbicides are degraded predominantly by soil microbes and hydrolysis (Vencill 2002). Certain soil factors including microbial composition and activity, moisture, organic matter, pH, temperature, and soil texture have shown to influence the persistence of herbicides (Ayeni et al. 1998). Especially under conditions of drought and/or cool temperatures these herbicides have the potential to persist past the season of application. The objective of this study was to determine the extent to which ALS inhibiting herbicides interact and influence phytotoxicity on sensitive rotational crops when applied sequentially.

## Materials and Methods

Three Saskatchewan locations were selected: Saskatoon, Melfort, and Scott, with the experiment starting in 2002 and repeated again starting in 2003. The experiment was set up as an RCBD with four replications of ten treatments. In the first year of the experiment all the treatments were seeded to peas (*Pisum sativum* L. 'Swing'), with treatments one through five being sprayed with a non-residual herbicide and six through ten being sprayed with imazamox/imazethapyr. In year two all the treatments were seeded to wheat (*Triticum aestivum* L. 'Eatonia') with treatments one and six sprayed with a non-residual herbicide, two and seven with imazamethabenz, three and eight with flucarbazone-sodium, four and nine with sulfosulfuron, and five and ten with florasulam. Between the second and third year growing seasons soil samples were taken from each treatment. In the third year all treatments were seeded to Roundup Ready<sup>tm</sup> canola (*Brassica napus* L. 'DKL 3455') and sprayed with a non-residual herbicide.

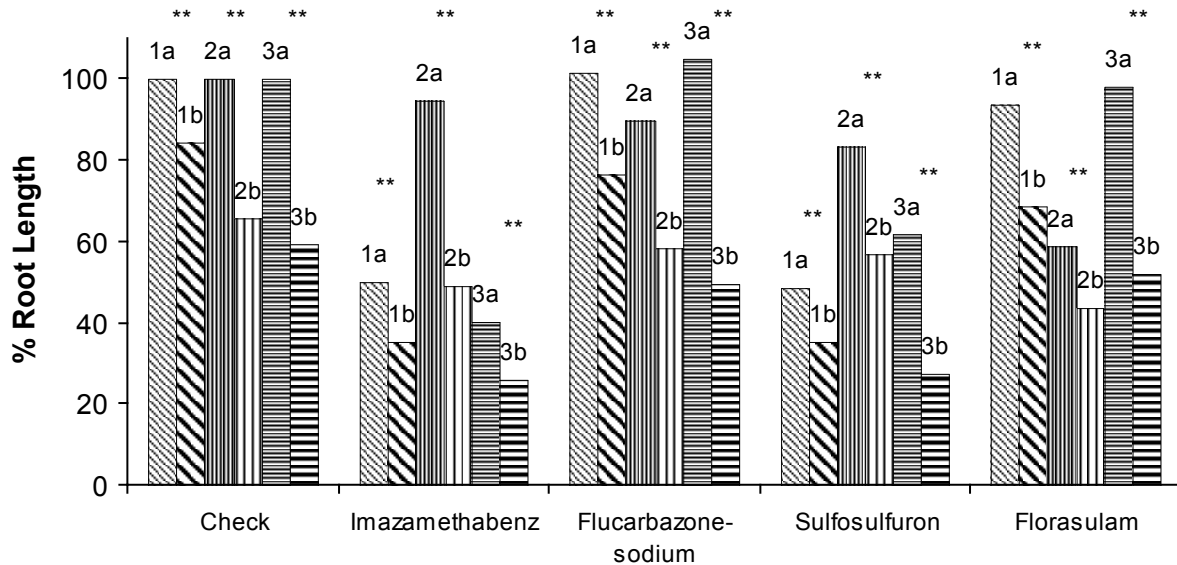
The soil samples were air dried and passed through a 2mm sieve. These soils were then used to perform a root inhibition bioassay to test for residual herbicides (Eliason et al. 2004). Oriental mustard (*Brassica juncea* L. 'Cutlass') was selected for the residual herbicide root length inhibition bioassays. The seeds were pre-germinated for 24 hours prior to seeding. For each field treatment 100 g of soil was measured and placed into 6 Styrofoam cups. The soil was then wetted to 80% water holding capacity. Five pre-germinated seeds of similar size and radicle protrusion were selected and placed into the Styrofoam cups, covered with a small amount of soil and lightly packed. The soil was covered with plastic beads to reduce evaporation losses. The cups were wetted to field capacity, randomized, placed under a fluorescent canopy, and covered with a plastic sheet for 24 hours. On the fifth day after seeding, the plants were manually removed from the soil and the root lengths were measured.

In order to determine if the interaction between two different herbicide residues in the soil is synergistic, additive, or antagonistic, the observed values need to be compared to expected values generated from Colby's equation (Colby 1967). Colby's equation states that  $E = (XY)/100$ , where E is the expected root length as a percent of the untreated check in the presence of 2 combined herbicides, X is the root length as a percent of the untreated check in the presence of herbicide A, and Y is the root length as a percent of the untreated check in the presence of herbicide B. When the expected root length is compared to observed root length as a percent of the untreated check, the type of interaction can be interpreted. If observed root length is less than the expected root length there is a synergistic interaction, if observed is equal to expected there is an additive interaction, or if observed is greater than expected there is an antagonistic interaction.

## Results and Discussion

The measurement of herbicide interactions for the field trials was via application of the root inhibition bioassay. In the field trial soil samples, the bioassay could detect soil residues from all five of the tested herbicides. In all cases the combined residues of imazamox/imazethapyr and either imazamethabenz, flucarbazone-sodium, sulfosulfuron, or florasulam resulted in greater root length inhibition than these four herbicides alone (Fig. 1). When all the measurements were

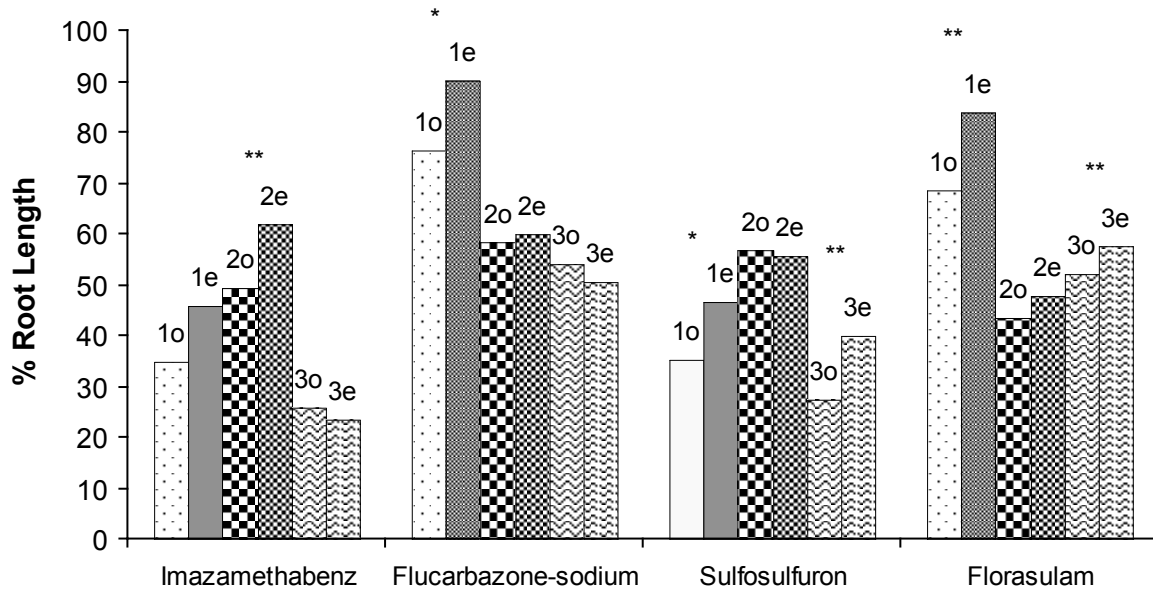
taken and both repeats were combined for each location, it was determined that there were significant differences between each combination of the year 2 herbicide alone and with the same treatment along with imazamox/imazethapyr. This data shows that many of these herbicides can persist past the season of application, and in the case of imazamox/imazethapyr it can persist into the second season after application. This implies that there is the potential for damage to susceptible crops in seasons after the application of residual herbicides, and an even greater potential if there were two applications of residual herbicides in successive years.



**Figure 1:** Average root length as a percent of the untreated check in an oriental mustard root inhibition bioassay using soil samples from the field trials. 1a are trials from the Saskatoon site that had no residual herbicide in the first year, while 1b had imazamox/imazethapyr the first year. 2a and 2b are Melfort trials that had no residual herbicide and imazamox/imazethapyr the first year respectively. The Scott trials are represented by 3a and 3b with treatments the same as at the other locations. Columns with the same site number and asterisks are significantly different.

The root inhibition bioassay results were examined using Colby's equation. The observed interactions between the imazamox/imazethapyr residues and the residues from the other four herbicides often varied from the expected values generated (Fig. 2). Therefore the interactions of the herbicides in the field trials appear to be additive in some soil types, while synergistic in terms of phytotoxic effects in other soil types. There were synergistic interactions between imazamox/imazethapyr and imazamethabenz at the Melfort site, imazamox/imazethapyr and flucarbazone-sodium at the Saskatoon site, imazamox/imazethapyr and sulfosulfuron at both the Saskatoon and Scott sites, and imazamox/imazethapyr with florasulam at both the Saskatoon and Scott sites. The remaining observed versus the Colby's calculated expected combinations at these sites were determined to be non-significant and therefore are additive in nature. The Saskatoon location appeared to show more instances of synergistic interactions in the root inhibition bioassay measurements, as compared to the Melfort site. In terms of herbicides being

more likely to have synergistic interactions, imazamox/imazethapyr with sulfosulfuron and florasulam had synergistic reactions in two site locations each. This data shows that there is the potential for herbicides to have synergistic interactions in the soil, however this does not appear to happen in all cases or with any predictability.



**Figure 2:** Average root length as a percent of the untreated check calculated from the oriental mustard root inhibition bioassays applied to soil samples from the Saskatoon, Melfort, and Scott locations, labeled 1,2, and 3 respectively after an application of imazamox/imazethapyr the first year and a residual wheat herbicide the second. The actual values are labeled with an o, while e stands for the expected value that was derived from Colby's equation (Colby 1967). Significant differences between paired columns with the same site number is indicated by asterisks, one for a p value <0.05 and two for a p value <0.001.

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