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Application of a Laboratory Bioassay for Assessment of Bioactivity of ALS-inhibiting Herbicides in Soil

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1. Introduction

Acetolactate synthase (ALS) herbicides inhibit the biosynthesis of branched chain amino acids (valine, leucine and isoleucine) in sensitive plants. The ALS-inhibitor group of herbicides includes sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinyl oxybenzoates , and sulfonylamino carbonyl trizolinones. They control a wide spectrum of broadleaf weeds and grasses and are commonly used in cereal and pulse crops, soybean and rice. Tolerant plants rapidly metabolize ALS-herbicides to an inactive product while sensitive plants show little or no metabolism of ALS-herbicides (Sweetser et al., 1981). ALS inhibition is a biological pathway that exists only in plants and not in animals, and therefore the ALS-inhibiting herbicides are considered to be safe (Colborn & Short, 1999). Because of the very high plant toxicity of ALS-inhibiting herbicides to susceptible plants, the application rates of these herbicides are remarkably low, typically between 3 to 150 g ai/ha (Senseman, 2007) making these herbicides environmentally attractive. The bioavailability of ALS-herbicides to plants is soil dependent, and the efficacy in weed control may decrease in soils of high organic matter and clay content and low pH. Dissipation of ALS-herbicides varies greatly with environmental conditions, soil characteristics and type of herbicide. Although the half-lives are relatively short, the small residual quantities remaining in soil may be of agronomic concern due to the high potency of these herbicides at low concentrations. The expected levels of soil residual ALS-inhibiting herbicides one year after application are at or below one part per billion concentrations. Addressing concerns regarding possible damage to successive crops requires the ability to detect extremely low concentrations of these herbicides in soil.

2. Plant bioassay techniques

Herbicide residues in soils can be determined by plant bioassays or by chemical methods. Chemical methods are specific, sensitive and quantify the total amount of herbicide residue in soil (Klaffenbach & Holland, 1993; Galletti et al., 1995; Stout et al., 1997; Szmigielska et al.,

1998; Smith, 1995). However, they may be costly, requiring extraction solvents and sophisticated analytical equipment, and can be time consuming as well. Plant bioassays are simple, inexpensive, and measure a phytotoxic portion of soil residual herbicide which typically varies with soil type and plant species. Also, because bioassays are non-specific, the effect of all residual herbicides present in soil is measured by bioassays (Johnson et al., 2005). Parameters that are frequently assessed in plant bioassays are root or shoot length, fresh or dry weight of roots or shoots, leaf area or plant height, visual estimation of plant injury, physiological and morphological effects such as photosynthetic activity, water consumption, or chlorosis (Horovitz, 1976). These measurements are assessed relative to a control sample which is needed because of the variation in plant growth in soils of different properties. Therefore, having a control soil that is identical in properties to the treated soil is considered essential for accuracy of a bioassay.

Various plant species have been used in bioassays for the determination of ALS- herbicides in soil, primarily using root measurements because of the inhibiting effect of ALS-herbicides on cell division at the root tips of susceptible plants. Some of the crops that have been used are corn for chlorsulfuron (Anderson & Humburg, 1987; Groves & Foster, 1985; Hsiao & Smith, 1983; Morishita et al., 1985), sunflower for MON-37500 and triasulfuron (Hernández-Sevillano et al., 2001), lentil for metsulfuron (Szmigielska et. al., 1998), and canola for imazethapyr (Szmigielska & Schoenau, 1999). Eliason et al. (2004) reported a sensitive bioassay using oriental mustard (*Brassica juncea* L.) as an indicator plant for flucarbazone. The mustard root bioassay was further improved by Szmigielski et al. (2008) and was used in Canadian prairie soils for investigation of other ALS-inhibiting herbicides that included imazamox-imazethapyr, sulfosufuron, florasulam, pyroxsulam and thiencarbazone. In this bioassay, oriental mustard plants are grown in 50 g soil. Soil is wetted to 100% field capacity, hand-mixed and transferred to a 2-oz Whirl-Pak bag. Subsequently, the soil in the Whirl-Pak bag is gently packed to form a layer that is approximately 8 cm deep and 1 cm thick (Fig. 1).

Fig. 1. Mustard root length bioassay performed in Whirl-Pak bags.

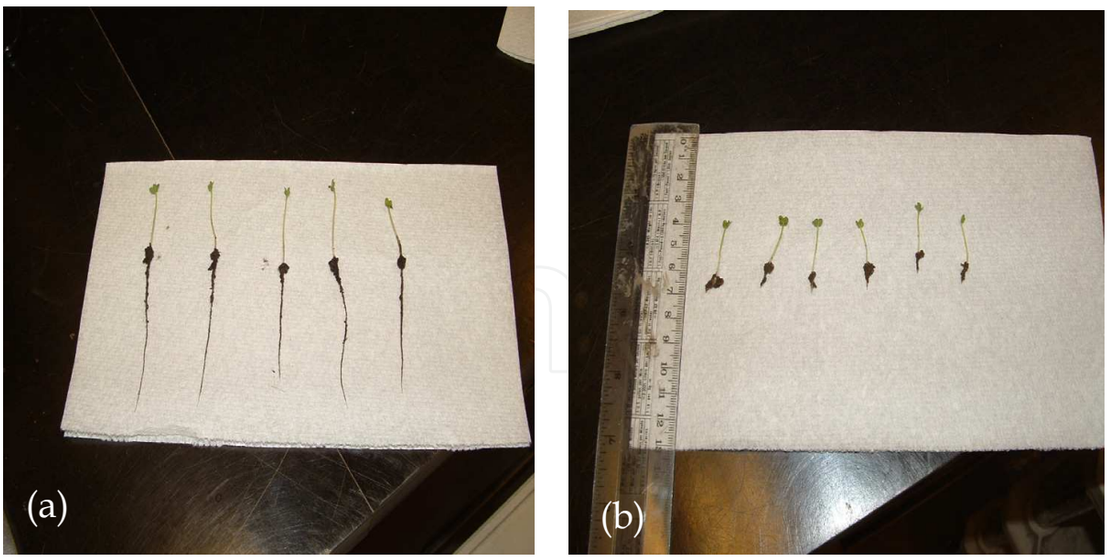

Fig. 2. (a) Mustard plants grown in control (uncontaminated) soil; (b) Mustard plants grown in treated (contaminated) soil.

Six oriental mustard seeds are planted per bag and plants are grown for three days in a fluorescent canopy. Plants are harvested after opening the bag and washing the soil away from the roots with water, and the length of roots is measured with a ruler. Root lengths in soils free of ALS-inhibiting herbicides are consistent among soils, and are in the approximate range of 7 cm ± 1 cm. A root length of 6 cm or less is considered to be indicative of herbicide residue present in the soil (Fig. 2). Because this bioassay is completed in three days, the reduction of root length is primarily due to the herbicide presence in soil as the effect of the nutrient status of the soil is minimized.

3. Effect of soil properties on herbicide phytotoxicity

Soil characteristics have a major influence on the bioavailability of ALS-inhibiting herbicides mainly because they affect sorption of herbicides to soil constituents. Most important properties in this regard are soil organic matter content, pH and texture. For studies of the relationships between herbicides, soil properties and plant species, dose-response curves based on a log-logistic regression model are frequently used (Seefeldt et al., 1995):

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

where y = plant response, x = herbicide concentration, C = lower limit of log-logistic curve, D = upper limit of log-logistic curve, I_{50} = concentration corresponding to 50% plant growth inhibition, and b = slope of the curve around the I_{50} value. I_{50} values can be estimated from the dose-response curves and can be used for examining relationships of herbicide phytotoxicity with factors such as soil characteristics, plant species or type of herbicide.

Organic matter in the form of humus is colloidal in nature with a highly reactive surface containing many functional groups capable of binding herbicide molecules. Reduced phytotoxicity of flucarbazone (Eliason et al., 2004; Geisel et al., 2008), imazethapyr (Szmigielska & Schoenau, 1999), metsulfuron (Szmigielska et al., 1998), pyroxsulam and thiencarbazone (Szmigielski A.M., unpublished) in Canadian prairie soils of high organic

matter content was explained by increased herbicide sorption. Using mustard root bioassay, dose-response curves were constructed for these herbicides and the I_{50} values were estimated. An example of varying dose-response curves with soil organic carbon content for pyroxsulam in Canadian prairie soils is shown in Fig. 3. Correlations of the I_{50} values with soil properties revealed that phytotoxicity of these herbicides was primarily related to soil organic carbon. However, soils used in the above studies had a broad range of organic carbon content but a relatively narrow range of soil pH. The narrow range of soil pH might have limited an assessment of the effect of soil pH on phytotoxicity of ALS-herbicides, as herbicide sorption to soil surfaces has been reported to be also pH-dependent. ALS-inhibiting herbicides are weak acids with pKa values in an approximate range of 3 to 5 (Senseman, 2007); therefore in soil solution at pH lower than the pKa value, these herbicides exist predominantly in nonionic form, whereas at pH values higher than the pKa, they are ionized. When these herbicides are in anionic form, their solubility in water increases and higher herbicide concentration is present in soil solution resulting in higher herbicide phytotoxicity. Soil pH also affects the ionic charges of the organic matter and clay colloids, with higher pH increasing the negative charge. Therefore, soil adsorption of weakly acidic herbicides generally decreases as soil pH increases due to repulsion of the herbicide anions from the negatively charged organic and clay surfaces. The relationship of herbicide adsorption and soil pH has been shown for many ALS-inhibiting herbicides including chlorsulfuron (Mersie & Foy, 1985), imazaquin and imazethapyr (Renner et al., 1988; Che et al., 1992; Loux & Reese, 1992; Goetz et al., 1986), imazapyr (Wang & Liu, 1999; Wehtje et al., 1987), and chlorimuron (Goetz et al., 1989). The effect of clay content on herbicide bioavailability is similar to the effect of organic matter in that the high surface area of clay can increase herbicide sorption and may further reduce herbicide bioavailability.

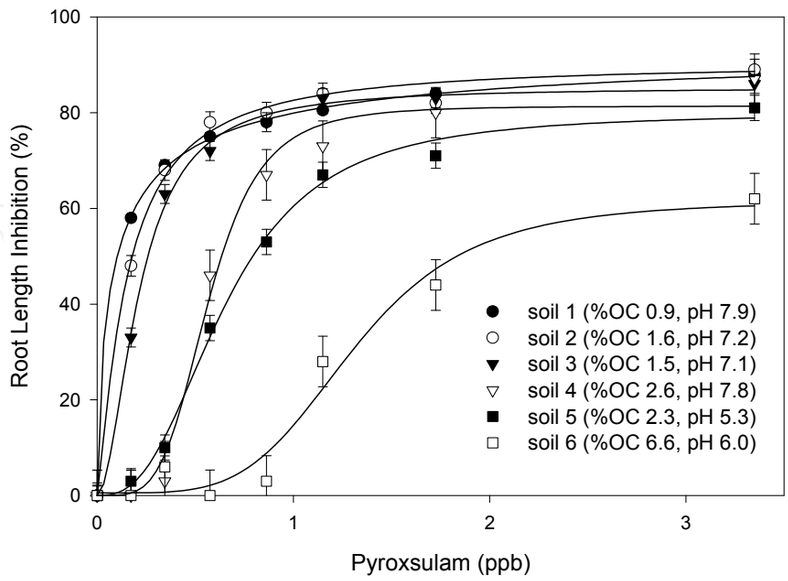

Fig. 3. Dose-response curves for pyroxsulam in Saskatchewan (Canada) soils as determined by the mustard root length bioassay under laboratory conditions.

The reduced phytotoxicity associated with high organic carbon and clay content and low soil pH may result in decreased efficacy of ALS-inhibiting herbicides. However, these soil characteristics may contribute to minimizing the injury to rotational crops by lowering the phytotoxicity of ALS-herbicide residues remaining in soil one year after application.
Because soil properties vary within the farm field landscape, the bioavailability of ALS-herbicides is affected by field topography (Schoenau et al., 2005). Studies of metsulfuron (Szmigielska et al., 1998), imazethapyr (Szmigielska & Schoenau, 1999) and flucarbazone (Eliason, 2003;) in Canadian prairie soils revealed that in lower slope positions, herbicide phytotoxicity was decreased as compared to the upper slope positions in the same farm field (Fig. 4). Reduced phytotoxicity in the lower slope soil is explained by the higher organic matter and clay content and lower pH compared to the upper slope soil. Thus, potential landscape effects on the phytotoxicity of ALS-herbicides should be taken into consideration when herbicides are applied to fields of variable topography.

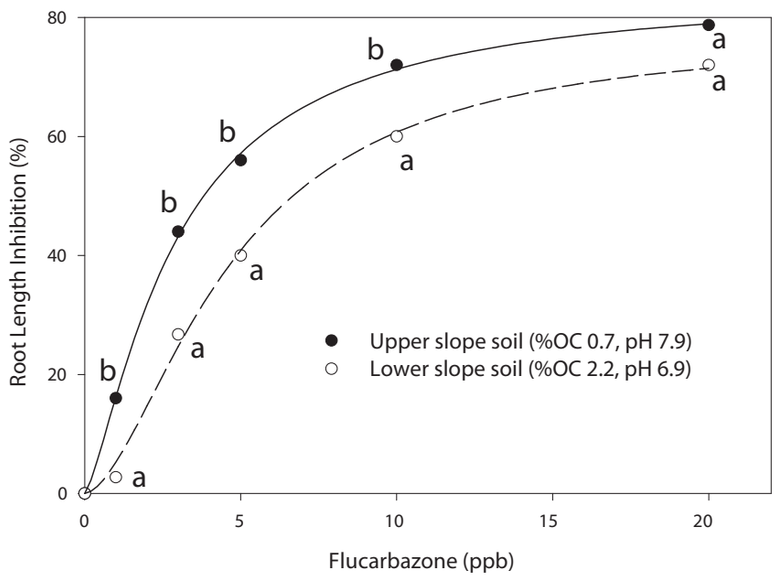

Fig. 4. Effect of landscape positions on phytotoxicity of flucarbazone in a soil from southern Saskatchewan (Canada); means for each flucarbazone concentration followed by the same letter are not significantly different at $p \leq 0.05$.

4. Herbicide dissipation under laboratory and field conditions

Herbicide dissipation in soils is influenced by environmental conditions and soil properties (Walker, 1991). The two primary mechanisms of ALS-herbicide dissipation are microbial degradation and chemical hydrolysis. Both these processes are dependent on soil water and temperature with faster dissipation occurring in moist and warm soils, particularly for herbicides that dissipate mainly through microbial processes (Beckie & McKercher, 1989; Joshi et al., 1985; Walker & Brown, 1983; Brown, 1990). Furthermore, experiments showed that degradation of ALS-herbicides is faster in non-sterile, microbially active soils than in sterile soils. The sterile degradation is slower and is due solely to chemical hydrolysis (Joshi et al., 1985).

Soil properties such as organic matter content, soil pH and texture play an important role in the dissipation of ALS-herbicides. High organic matter and clay content decrease the dissipation rate by limiting the amount of herbicide available in soil solution for biodegradation because of the adsorption process. A low soil pH tends to increase the persistence of ALS-herbicides as herbicide's adsorption to soil particles is enhanced under acidic conditions. Also, at low soil pH the ALS-herbicides are likely to exist as neutral molecules which are less soluble in water than ionized molecules, thus further reducing the amount of herbicide available for degradation.

Generally, the pattern of dissipation of ALS-herbicides is biphasic both under laboratory and field conditions (Brown, 1990; Loux et al., 1989; Hill & Schaalje, 1985; LaFleur, 1980; Eliason et al., 2004). In the biphasic dissipation process, initial rapid dissipation is followed by a slower dissipation rate at lower residual concentrations. In the rapid stage of dissipation, the readily available portion of the herbicide is degraded, whereas in the slow stage, the remaining molecules are tightly adsorbed to the soil particles and are less available for dissipation. (Zimdahl & Gwynn, 1977). A two-compartment (bi-exponential) regression model is frequently used to describe dissipation of ALS-herbicides in soil (Hill & Schaalje, 1985):

$$C = C_0 \exp[-(k_s + k_r)t] + C_0 \frac{k_r}{k_s + k_r - k_d} \{(\exp[-k_s t] - \exp[-(k_s + k_r)t]\} \quad (2)$$

where C = herbicide concentration remaining in soil after time t, C_0 = initial herbicide concentration, k_d = dissipation rate constant, k_s = surface loss rate constant, and k_r = retention rate constant.

Herbicide half-lives can be estimated from the dissipation curves and their relationships with parameters such as environmental conditions or soil characteristics can be examined, as the half-lives of ALS-herbicides vary greatly with temperature, moisture, soil type, and also with herbicide type. Goetz et al. (1990) found half-lives for imazethapyr to range from 192 to 318 days in a silty-clay soil and from 78 to 270 days in a silty-loam soil both incubated at different soil moisture and temperature conditions. Chlorsulfuron half-life was longer at lower temperature (229 days at 10 C and 62.5 days at 40 C) and varied with soil pH (88.5 days at pH 6.2 and 144 days at pH 8.1) (Thirunarayanan et al., 1985). For amidosulfuron half-life values ranged from 14 days in a loamy sand incubated at 30 C to 231 days in a clay incubated at 10 C (Smith & Aubin, 1992). Beckie & McKercher (1989) reported half-lives for DPX-A7881 herbicide to increase from 33 to 214 days in a soil with pH adjusted from 5.5 to 8.1. Using the mustard root bioassay, Eliason et al. (2004) studied flucarbazone dissipation in Canadian prairie soils of contrasting properties under laboratory conditions of 25 C and moisture content of 85% field capacity. Flucarbazone half-lives ranged from 6 to 110 days and half-lives were significantly correlated with soil organic carbon with longer half-lives in soils of higher organic carbon. Johnson E.N (unpublished) found that the half-life for flucarbazone dissipation under field conditions in a loamy textured Dark Brown Chernozem was approximately 11 days and was not dependent on flucarbazone application rate (Fig. 5).

Although half-lives of ALS-herbicides are generally short under optimal conditions of moisture and temperature, under conditions of drought and/or cold weather the ALS-inhibiting herbicides may persist in soil and may carry over to the next growing season at levels that cause injury to rotational crops.

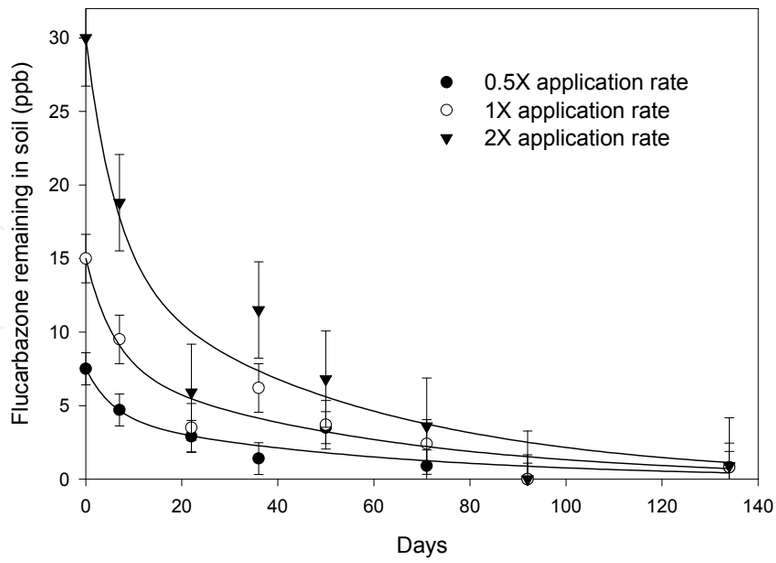

Fig. 5. Field dissipation of flucarbazone in a loamy textured Dark Brown Chernozem soil (organic carbon 2.3%, pH 5.3) in central Saskatchewan (Canada) at different application rates.

5. Predicting potential carry-over injury to subsequent crops

For rotational crop damage to occur, a herbicide must have sufficient persistence in soil, must be available to plants at a phytotoxic level, and the rotational crop must be susceptible to the residual concentration of the herbicide remaining in soil at the time of planting (Hartzler et al., 1989). Because of the high potency of the ALS-herbicides, the minuscule residual levels may remain active and may exert an effect on sensitive crops. Moyer et al. (1990) reported that when chlorsulfuron was applied in wheat on alkaline soils of relatively low organic matter in Alberta (Canada) at the recommended level of 40 g ai/ha, successive crops were affected; the required time for recropping barley, canola, pea, bean, flax, potato, alfalfa, sugar beet and lentil ranged from 2 to 7 years. In another study in Alberta (Moyer & Esau, 1996), imazamethabenz reduced the yield of sugar beet seeded 1 year after application, while imazethapyr application increased the risk of yield loss of flax, corn, meadow bromegrass, mustard, sunflower, timothy, wheat, canola, sugar beet and potato seeded between 1 to 3 years later. Crops that were reported as very sensitive to sulfonylurea herbicides are lentil, sugar beet and turnip, and as sensitive are alfalfa, canola, corn, flax, garden cress, lettuce, mustard and sunflower (Smith, 1995; Moyer & Hamman, 2001).

Typically, ALS-herbicides remaining in soil one year after application are at very low concentrations and are difficult to detect by analytical methods. Therefore plant bioassays are frequently used for the determination of bioactive herbicide residues. For the results of a bioassay to be most reliably interpreted, plant response in contaminated soil should be compared to the plant response in a control (uncontaminated) soil. However, having a control soil sample identical in properties to treated soil may be a problem especially in testing farm field soils. To overcome this difficulty, a designated non-contaminated soil may be used as a control as described by Watson & Checkel (2005). In their method the target

crop, a check crop and a sensitive crop are planted in both the soil submitted for testing and a check soil, and the symptoms consistent with herbicide damage are reported.

In the mustard root length bioassay reported by Szmigielski et al. (2008), root lengths in non-contaminated soils are uniform among soils, thus the need for a control sample is eliminated. This method is not intended to determine ALS-herbicides quantitatively but rather to "red-flag" the soils for potential presence of herbicide residues. For flucarbazone that was applied in replicated field trials in western Canada, comparison of the results of the mustard root bioassay and chemical analysis with the yield of subsequent crops revealed that the bioassay was a better predictive tool for yield reduction than chemical analysis (89% and 27% agreement, respectively). While these results showed that the mustard root bioassay provides a good level of accuracy in predicting injury, 6% of the results were false positive and 5% were false negative. False positives (flucarbazone detected by the bioassay but no crop injury observed in the field) pose no risk of crop damage; however they would restrict re-cropping options. False negatives (no flucarbazone detected by the bioassay but crop injury observed in the field) could represent significant crop damage and loss of income for the grower.

Interpreting bioassay results and making re-cropping recommendations is a complex task and should be approached with caution (Watson & Checkel, 2005). Soil field sampling is critical for the bioassays because a single sample may not be representative of the whole field unless a sample is carefully obtained either by using a composite sample from different locations in the field or by sampling different parts of the field separately. Factors such as soil characteristics (organic matter content, pH and texture), farm field topography, previous herbicide use, crop history and weather conditions should be considered together with the bioassay results when determining which crops to grow in the following year.

6. Herbicide interactions after successive field applications

Repeated applications of ALS-inhibiting herbicides may result in interactions of the residues existing in soil from previous years with a herbicide that is applied in succession. Combined effect of two or more herbicides may be additive if actual and expected responses are similar, may be synergistic if the actual response is greater than the expected, and may be antagonistic if the actual response is less than the expected (Colby, 1967). Because many ALS-herbicides have residual properties, a potential for interactions exists with successive applications.

Limited research has been conducted on the crop response from repeated applications of the same ALS-herbicide or different ALS-herbicides. Moyer & Hamman (2001) reported that residues of MON 37500 herbicide combined with either imazethapyr or metsulfuron, or trisulfuron resulted in additive injury to sugar beet. Johnson et al. (2005) reported that the application of ALS-herbicide can predispose the following crop to higher levels of phytotoxicity from postemergence ALS-herbicide application.

Interactions of imazamethabenz, flucarbazone, sulfosulfuron and florasulam in combination with imazamox/imazethapyr in western Canadian soils were investigated in laboratory experiments (Geisel, 2007) and field trials (Geisel et al., 2008). In the laboratory experiments, soils were amended with individual herbicides and combinations of herbicides, and their effect on mustard root length inhibition was measured. The interactions between the investigated herbicide combinations were additive: the expected (calculated) responses and the actual (observed) responses to each pair of herbicides were the same, as seen in Fig. 6 for the imazamox/imazethapyr and florasulam combination.

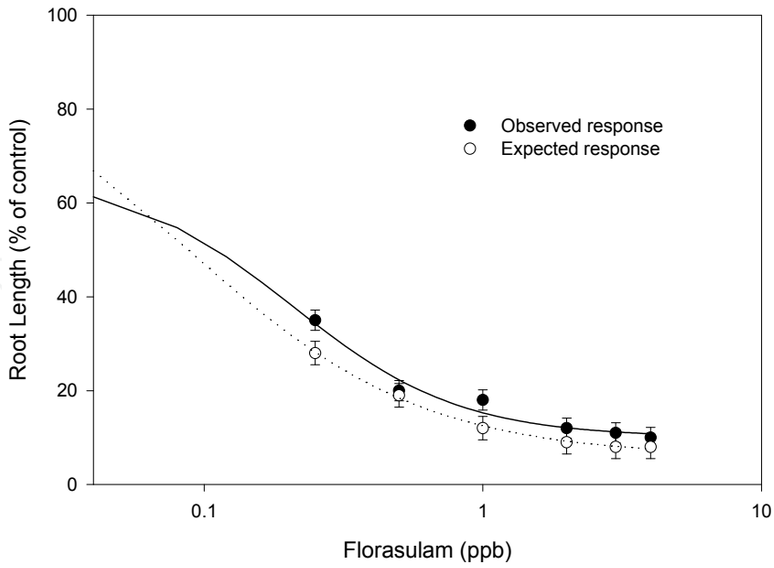

Fig. 6. Mustard root response (as % of control) for florasulam in combination with imazamox/imazethapyr in a Dark Brown clay textured Chernozem soil from southern Saskatchewan (Canada).

In the field trials, herbicides were applied sequentially over the course of 2 years; in the first year only imazamox/imazethapyr was applied and in the second year imazamethabenz, flucarbazone, sulfosulfuron or florasulam was added to the plots. All plots were sampled in the third year before the next crop was seeded and the herbicide residues were determined with the mustard root bioassay. Similar to observations for the laboratory experiments, in the field trials herbicide residue combinations showed additive injury.

As application of ALS-herbicides in successive years is becoming a frequent practice, producers need to be aware of the fact that the bioactivity of the herbicide residues persisting in soil from previous years may add to the bioactivity of the applied ALS-herbicide and that this practice may result in increased risk of injury to subsequent crops that are sensitive to both herbicides.

For some herbicides, repeated applications may also lead to enhanced degradation. Enhanced degradation occurs when a herbicide is applied to a field that received a prior treatment of the same herbicide (Roeth et al., 1989; Walker & Welch, 1991). It is believed that enhanced degradation is a result of microbial adaptation which consequently increases the rate of microbial activity. Enhanced degradation helps in minimizing the concentration of residual herbicide that may persist in soil to the following season. However, it may also result in reduced weed control in the year of application (Johnson et al., 2005).

7. Conclusions

Plant bioassays are an effective tool in research and in soil testing because they detect the phytotoxic amount of herbicide present in soil. A laboratory bioassay based on the root length inhibition of oriental mustard for detection of ALS-herbicides is simple and quick; it is completed in three days and uses only 50 g of soil per replication. With measurements generally requiring 4 replications, the total amount of soil needed to perform the bioassay is 200 g. Root development in a Whirl-Pak bag is not restricted as the bioassay is completed

before the roots grow to the bottom of the bag, therefore root reduction is only due to the presence of an ALS-herbicide. Recovery of roots from soil is very easy because soil is removed from roots by a gentle stream of water after the bags are cut open, and roots do not get damaged or broken before being measured. Consequently, the results of the bioassay are reproducible (coefficient of variation of approximately 6% based of 4 replications) and sensitive (detection limit of approximately 1 ppb).

The mustard root bioassay was successfully used to examine activity and behavior of several ALS-inhibiting herbicides in soils of the Canadian prairies. Phytotoxicity and persistence of ALS-herbicides was found to be mainly affected by organic matter content in prairie soils: higher organic matter content results in decreased phytotoxicity and in slower dissipation. Thus efficacy in weed control in the season of application will be lower and potential for herbicide carry-over to the next season will higher in soils of high organic carbon. A study of carry-over injury showed that a mustard root bioassay is a useful technique for predicting potential crop damage due to residual ALS-herbicides. Laboratory and field evaluations of the effects of combinations of different ALS-inhibiting herbicides in soil showed that interactions among residues in prairie soils are additive.

8. References

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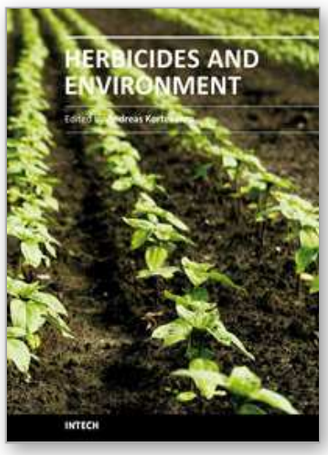

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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