Using a Plant Bioassay to Detect Herbicide Residue

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Abstract

Soil residual herbicide activity has been investigated since the early 1960s. One objective has been to provide producers with specific re-crop recommendations. Numerous plant bioassays have been developed to meet this need. The Alberta Research Council (ARC) has been providing a bioassay service to detect symptoms consistent with herbicide carryover since 1986. The objective of this paper is to describe our bioassay methodology and to provide basic information arising from our experience. Soil samples suspected of containing active residues are submitted to the ARC. Once the soil sample is received, the target crop and a sensitive species are planted in both the submitted soil and a check soil, known to be herbicide-free. Samples are evaluated for initial severity of symptoms and recovery over time. Symptoms consistent with herbicide carryover were found in 77% of all samples submitted. The most common crop-herbicide type combination requested is canola-imidazolinone. The primary limitation of bioassays is that damage in the bioassay may not reflect yield loss in a producer's field. However, bioassays are the only risk-management tool available to producers and can detect the presence of residues below chemical detection thresholds.

Introduction

The effects of residual herbicides on subsequent crops has been investigated since the early 1960's (Chubb 1963). Early research used field experiments (Wicks et al. 1969) to detect herbicide carryover. This research offered general recommendations, recognizing that herbicide carryover was affected by crop sensitivity, soil texture, organic matter and precipitation (Wicks et al. 1969). Producers' need for specific re-crop recommendations for specific fields was recognized (Anonymous 1977) and bioassays were developed to mitigate this problem (Anonymous 2004).

Bioassays to detect herbicide residual activity have examined root growth reduction in soil media (Eliason et al. 2002; Holloway et al. 1999) and petrie dishes (Jourdan et al. 1998), and whole-plant bioassays in soil media (Loux et al. 1989) and hydroponics (Sandín-España et al. 2003) for a number of herbicide-crop combinations. A common research goal has been to compare the bioassay with other methodologies to detect herbicide residues, thereby preventing yield loss. In general, this research has found that bioassays are suitable screening tests for herbicides employed at low rates and can be useful in detecting the presence of low levels of phytotoxic soil residues (Anonymous 2000).

The ARC has been providing a bioassay service to detect symptoms consistent herbicide residue since 1986 and has considerable expertise in this area. This service has been provided to producers, extension agronomists, chemical companies, and the research community. It has been used as a risk management tool to aid re-crop decisions by agronomists and producers and, to

detect symptoms consistent with residue after injury is observed on crops. The objective of this paper is to describe the bioassay methodology and provide information arising from our experience.

Materials and Methods

Sampling Fields

Soil analysis can provide detailed information on nutrients or herbicide residues. Soil analysis information useful in assisting interpretation of bioassay results include pH, organic matter, soil texture, and soil moisture (Bresnahan et al. 2002; Eliason et al. 2002; Loux et al. 1989; Moyer 1995). However, a small soil sample may not be representative of the whole field unless the sample is carefully selected (Rubem et al. 1999). It is important to have a full record of the field sample including information on location and topography (Rubem et al. 1999), crop history and agronomic practices (Jensen et al. 1995) and herbicide use history.

Two soil sampling strategies are commonly used, and either may be appropriate for a bioassay. Topographic soil sampling entails obtaining samples from eroded knolls, mid-slopes and low-lying areas and each soil sampling site can be subjected to a bioassay. This sampling system is useful for fields having rolling landscapes and can detect "hot spots" of carryover in the field as well as areas less likely to be affected. Our experience indicates this sampling methodology is most useful after injury has been observed. By contrast, random soil sampling avoids potential problem areas such as saline areas, poorly drained areas, and eroded knolls should not be sampled unless they represent a significant portion of the field. Accuracy is best preserved by submitting samples from 80-acre areas within a field. Each soil sampling site can be subjected to a bioassay. Agrologists and producers may find that one or both methods may be used on the same field.

Our sample requirements for a single bioassay are a minimum of 2 kg of soil per sample area to provide sufficient soil to conduct a bioassay with three plant species. A sampling depth of 0-7.5 cm for direct-seeded fields and 0-15 cm for tilled fields is based tillage effects (Berger et al. 1999). Check soil samples may also be submitted if a herbicide free area can be found, but all bioassays are conducted using a known herbicide-free ARC check soil. Soil samples should be collected and submitted prior to fall freeze to facilitate timely transmittal of results.

Bioassay method

Once the soil sample for the bioassay is received, the target crop, a check crop, and a sensitive species are planted in both the submitted soil and the check soil. Using glyphosate-tolerant canola (*Brassica napus* L.), testing for imidazolinone residues as an example (Figure 1), we would seed in the submitted and check soils: 1) glyphosate-tolerant canola (target crop), 2) imidazolinone-tolerant canola, and, 3) sugar beet (*Beta vulgaris* L.) as a sensitive species. Since imidazolinone-tolerant canola is not sensitive to imidazolinone, this approach ensures we detect bioavailable herbicide and do not confuse other symptoms, such as sulphur deficiency, pH response, or salinity, with herbicide activity. For other crop-herbicide combinations, different check and sensitive species are used. Samples are evaluated for initial severity of symptoms and recovery over time and this information is reported to the agronomist or producer. Mild injury may not include chlorosis or substantial growth reduction. Symptoms may be limited to short-term purpling and cupping of leaves in the target species with more severe symptoms present in the sensitive species. Severe injury (Figure 1) can include substantial chlorosis, purpling and cupping of leaves and

disruption of meristems. This can result in significant growth reduction and excessive lateral branch production in the target and sensitive species or both, the target and sensitive species.

Interpreting Bioassay Results

Since the ARC does not chemically evaluate the presence or concentration of specified herbicides, we do not state that these herbicides are present. Instead, we evaluate for "symptoms consistent with herbicide damage". Consequently, the ARC does not provide recommendations to agronomists or producers. Agronomists and producers receiving reports jointly make decisions based on the information provided and previous experience. Bioassays are most difficult to derive reliable recommendations from when they are not either completely "dirty" or completely "clean". Feedback from agronomists indicates that when we report severe symptoms on the target species (e.g. canola), the decision is undertaken not to plant that crop. In the Peace River area, which is relatively acidic, damage to sugar beet (sensitive species) indicates a canola recrop may not be suitable.

Figure 1. Bioassay showing severe damage to both the target (canola - left) and sensitive species (sugar beet – right).

Results and Discussion

The ARC, formerly the Alberta Environmental Center (AEC), has provided a residual bioassay service since 1986. Early sample submissions consisted largely of picloram (Tordon) and later chlorsulfuron (Glean). Since record-keeping commenced in 1999, approximately 900 samples have been received (Table 1). These samples have come primarily from agronomists, but since the drought of 2002, there has been a substantial increase in the number of samples coming from industry and research (Table 1).

Most samples are submitted to aid re-crop decisions, and interest has focused on predominantly on high-acreage, high-value crops. Approximately 63% of all samples received request canola as a target crop (Table 2). The next most frequently requested crop is peas, however, it is the requested target crop less than 10% of the time. Major crops, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), lentil (*Lens culinaris* Medik.), and tame oats (*Avena*

sativa L.) are also represented. In addition to these crops, bioassay have been requested for: 1) forage crops such as brome (*Bromus* spp.), fescue (*Festuca* spp), fescue/alfalfa (*Medicago sativa* L.) mixes, and timothy, 2) various bean crops (*Phaseolus* spp.) such as faba, navy, soy, and pink, 3) oilseeds such as sunflower (*Helianthus annuus* L.) and flax (*Linum usitatissimum* L.), and, 4) specialty crops such as canaryseed (*Phalaris canariensis* L.), coriander (*Coriandrum sativum* L.), corn (*Zea mays* L.), mustard (*Sinapsis alba* L. and *Brassica juncea* L.), potatoes (*Solanum tuberosum* L.) and sugarbeets. This represents a wide range of experience in inspecting and interpreting symptoms.

Table 1. Sample Sources and Number of Samples Received for Bioassay Service Since 1999.

Year	Agronomist	Industry	Research	Producer	Government	Other	Total
1999	24	-	6	-	-	-	30
2000	36	-	4	5	3	-	48
2001	51	-	-	13	3	-	67
2002	98	61	-	6	3	-	168
2003	229	15	-	31	2	1	278
2004	118	59	76	22	-	2	277
Total	556	135	86	77	11	3	868

Sample submissions have been received from a wide geographic range (Table 3). Most samples come from Alberta, excluding the South region, and Saskatchewan (Table 3). A considerable increase in samples from all areas was observed after the drought of 2002 (Tables 1-3). Fewer samples from southern Alberta may be attributable label restrictions and heightened awareness of the potential for herbicide soil residual activity due to: 1) drier conditions than are found in the central and northern parts of Alberta, and 2) research initiated in that part of the province (Moyer 1995). By contrast, Manitoba tends to receive more rainfall and herbicide soil residual activity should be less problematic.

Table 2. Target Species for Bioassay Service Since 1999.

Year	Canola	Peas	Wheat	Barley	Lentil	Tame Oats	Other	Total
1999	19	-	-	5	6	-	6	36
2000	18	2	1	5	4	1	16	47
2001	45	23	5	1	8	7	10	99
2002	77	9	12	13	-	6	14	131
2003	240	21	19	13	1	1	30	325
2004	76	9	16	9	-	1	7	118
Total[1]	475	64	53	46	19	16	83	756

1. Excludes missing data and some samples submitted by industry.

Sample submissions have requested tests for herbicide activity from Groups 2, 3, 4, 5 and 6, but have been dominated by group 2 herbicides (Table 4). More than 85% of samples received have requested testing for Group 2 herbicides and greater than 68% of these have requested imidazolinone activity (Table 4), generally using canola as the target species (data not shown). These results may arise from 2 factors. Firstly, imidazolinone herbicides were introduced in

Alberta relatively recently. Imazethapyr (Pursuit) was introduced to Alberta in 1995, followed by imazamox + imazethapyr (Odyssey) in 1998. Secondly, imidazolinone and sulfonylurea herbicides represent significant risk of residual activity under some conditions (Moyer 1995, Moyer and Esau 1996). Unknown herbicides (Table 4) frequently represent situations where a producer is renting, or planning to rent, land and has no cropping history to make re-crop decisions. In these cases multiple target species and herbicides maybe evaluated.

Table 3. Location of Sample Submissions Residual Bioassay Since 1999.

Year	BC	AB				SK	MB	Total
		South	Central	East Central	Peace			
1999	2		13	5	8	2	-	30
2000	2	3	8	11	20	-	-	44
2001	3	8	7	22	14	5	8	67
2002	-	11	27	9	17	34	9	107
2003	-	38	55	58	53	45	26	275
2004	1	6	14	19	22	29	16	107
Total[1]	8	66	124	124	134	115	59	630

1. Excludes missing data and some samples submitted by industry.

Symptoms consistent with herbicide soil residual activity have been found in approximately 77% of submitted samples. In 52% of samples submitted, the target species showed some herbicide damage. In addition, 25% of samples showed damage to the sensitive species, but not the target species. The remainder of samples showed no symptoms to either the target or sensitive species. These results suggest that producers expecting soil residual are likely to find it.

Table 4. Herbicides Tested With Bioassay Service Since 1999.

Year	Herbicide Group										Total
	2				3	4	5	6	Picloram	Unknown	
	IMI	SU[2]	IMI & SU	2,4							
1999	17	2	-	-	-	-	-	-	7	5	31
2000	23	4	5	-	-	4	-	-	-	12	48
2001	26	25	2	-	-	14	-	-	-	7	74
2002	69	26	9	3	3	3	1	-	1	1	116
2003	179	92	18	6	1	13	1	3	1	2	316
2004	69	29	-	4	1	10	1	-	-	1	115
Total[1]	383	178	34	13	5	44	3	3	9	28	700

1. Excludes missing data and some samples submitted by industry.
2. Includes sulfonylaminocarbonyltriazolinones and triazolopyrimidenes.

In conclusion, soil residual herbicide bioassays have both limitations and benefits. Limitations include that sampling may not adequately reflect whole-field variability. For example, agronomists and producers may sample in strips where overspray has occurred and a 2x concentration is present. Growing the sample out in a small pot or cup is not reflective of a field situation where roots have the opportunity to grow out of the herbicide layer. Finally, samples can fall in a gray area where there is damage to the sensitive species, but not the target species. In this

case reliable recommendations to producers may be difficult. Finally, when minor damage to the crop occurs, little is known about recovery and yield loss under different climatic and edaphic conditions.

Benefits of the bioassay are that whole-plant bioassays show biological effects of herbicides present, often at levels below chemical detection thresholds. They can be more useful than chemical detection methods due to interactions with soil organic matter, pH, soil moisture and soil texture. Finally, it is the only risk management tool available to producers at this time. Results from the bioassay should not be interpreted alone. Interpretation needs to include other tools and information such as label recommendations, rainfall restrictions, pH, organic matter, soil texture, and perhaps most importantly, producer and agronomist experience.

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