Ground-truthing The Soil Residual Herbicide Bioassay

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Abstract

The soil residual herbicide bioassay has been commonly used to determine the potential for crop injury following the use of residual herbicides. In spite of the bioassays use, there has been very little information available on the accuracy of the bioassay. In order to assess the level of accuracy, two independent studies were conducted; the first by AAFC, and the other by BASF. Existing sites containing soil residual herbicides were used, and field results on these sites were compared to the greenhouse bioassay result. The AAFC study showed low corroboration, and a high number of false positives, while the BASF study showed low corroboration and a high number of false negatives. Both studies indicated that the level of accuracy of the bioassay was low, and was not predictive of the result found in the field.

Introductions

Drought in the early part of the decade resulted in concerns with soil residual herbicide carryover. Periodically, herbicide carryover and injury to sensitive rotational crops has been problematic in the past with dinitroaniline herbicides (trifluralin, ethafluralin) and auxinic herbicides like picloram and clopyralid. More recently, the popular Group 2 ALS inhibitor herbicides such as the sulfonylureas (Sundance®), the imidazolinones (Assert®, Odyssey®, and Pursuit®), and the sulfonylamino carbonyltriazolinone (Everest®) herbicides have caused injury to sensitive rotational crops, particularly in dry locations with low organic matter, medium to coarse-textured soils. Predicting the breakdown of residual herbicides on the Canadian prairies is difficult due to the relatively short growing-season, and the unpredictable and highly variable weather. Agronomists and retailers need information and tools to assist growers in the planting decisions. Product labels are not always comprehensive enough to provide information on all re-cropping scenarios for growers. The Alberta Research Council (ARC) provides a fee-for-service plant bioassay that can detect herbicides in submitted soil samples. The information provided to the grower or agronomists are visual assessments of plant responses and no

recommendations are made. In spite of the popularity of the bioassay, there has been very little validation of the laboratory bioassay with results from field studies. The purpose of this study was to determine the level of accuracy of the laboratory bioassay, by comparing the bioassay results, to the results found in field studies.

Materials and Methods

Two independent field studies were conducted in Saskatchewan and Alberta in 2003-2005. The first study was conducted by Agriculture and Agri-Food Canada in collaboration with Alberta Agriculture, Food, and Rural Development. The second study was conducted by BASF Canada.

AAFC Field Study

The Agriculture and Agri-Food Canada studies involved the use of existing herbicide carryover field trials. Soil from the herbicide stacking trials at Scott, Melfort and Vanscoy were collected in May of 2004 (Johnson et al. 2004). The herbicide stacking trials were conducted in a field pea-spring wheat-canola sequence that commenced in 2002. Herbicide treatments are outlined in Table 1. All herbicides were applied at the 1.0 X label rate. Soil samples were taken from Treatments 1, 5, 8, and 9 at Melfort and Vanscoy, and Treatments 1, 3, 4, 5, 6, 8, 9, and 10 at Scott (one extra sample inadvertently taken from Treatment 7).

Table 1: Herbicides and rates applied in herbicide stacking trials (herbicide groups in parenthesis).

Treatment No.	Field Pea Herbicide (2002)	Spring Wheat Herbicide (2003)
1	Basagran ® (6)*	Horizon ® / Buctril-M ® (1, 6 & 4)*
2	Basagran ® (6)*	Assert ® (2)
3	Basagran ® (6)*	Everest ® (2)
4	Basagran ® (6)*	Sundance ® (2)
5	Basagran ® (6)*	Frontline ® (2)
6	Odyssey ® (2)	Horizon ® / Buctril-M ® (1, 6 & 4)*
7	Odyssey ® (2)	Assert ® (2)
8	Odyssey ® (2)	Everest ® (2)
9	Odyssey ® (2)	Sundance ® (2)
10	Odyssey ® (2)	Frontline ® (2)

* Non-group 2 / non-residual checks

Two responsive sites were chosen (Scott, Vanscoy) and one less responsive site was chosen (Melfort). Soil characteristics for the three sites are outlined in Table 2.

Table 2: Soil characteristics of sites used in AAFC field study.

Site	Soil Zone	S.O.M. (%)	pH	Texture
Scott	Dk. Brown	3.0	5.9	Loam
Vanscoy	Dk. Brown	5.3	7.1	Loam
Melfort	Thin Black	11.3	6.6	Clay

Soil sampling consisted of taking 4 to 5 cores from each plot and sampling to a depth of 7.5 cm. The cores were mixed from each plot, bagged and then immediately frozen. At least 1 kg of soil was collected from each treatment. Treatments from each replicate were bagged separately. In the fall, the frozen samples were driven to the Alberta Research Council in Vegreville, AB for the plant bioassay. In total, 61 samples were submitted from these three locations.

In 2004, the sites were seeded to Roundup Ready canola (cv. LG3455). Visual injury ratings were taken 7, 14, and 28 days after canola emergence. Canola yields were also taken.

Soil samples were also taken from a study where different rates of imazethapyr (Pursuit ®) were applied in the fall of 2001 and seeded to chickpea and lentil (plots were split) in 2002. The plots were seeded to wheat and barley in 2003 and Roundup Ready canola (cv. LG3455) in 2004. Soil samples were taken in the spring of 2004 from plots that had received 0 (check), 0.25, 0.33, and 0.5 X rate of Pursuit ® in the fall of 2001, or a 0.5 X rate of Pursuit ® in the spring of 2002. In total, 21 samples were submitted from this trial as one extra plot was inadvertently sampled.

Bioassays at the Alberta Research Council were conducted using sugar beet, canola, and Clearfield canola as indicator crops. Further information on the bioassay can be obtained at:

www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex4703?opendocument

BASF Field Study

The study conducted by BASF involved using existing rotational cropping studies that were established on 10 sites in 5 locations in 2002 and 2003 (Table 3). The trials were established on 8 X 12 m plots, planted to an imidazolinone tolerant crop (Clearfield wheat), and sprayed with three different imidazolinone herbicides at the 1x and 2x rates (Table 4). Soil samples were taken for bioassay analysis in the spring of 2003 and 2004, prior to seeding.

Table 3: Soil characteristics of sites used in BASF study.

Site	S.O.M (%)	pH	Texture	Soil Zone
Nisku, AB	10.0	5.8	Loam	Black
Morden, MB	5.2	7.1	Clay Loam	Black
Stirling, AB	2.0	7.2	Sandy Loam	Dk. Brown
Vanscoy, SK	7.0	6.1	Silty Clay	Dk. Brown
Estlin, SK	3.2	8.1	Clay	Brown

Table 4: Herbicides and rates applied in rotational cropping studies. BASF studies.

2002 Clearfield Wheat	Rate	Units	Bioassay Sampling
Assert	1.67	L/ha	2003, 2004
Assert	3.33	L/ha	2003, 2004
Odyssey	0.043	Kg/ha	2003, 2004
Odyssey	0.086	Kg/ha	2003, 2004
Imazamox	0.167	L/ha	2003, 2004
Imazamox	0.333	L/ha	2003, 2004

2003 Clearfield Wheat	Rate	Units	Bioassay Sampling
Assert	1.67	L/ha	2004
Assert	3.33	L/ha	2004
Odyssey	0.043	Kg/ha	2004
Odyssey	0.086	Kg/ha	2004
Imazamox	0.167	L/ha	2004
Imazamox	0.333	L/ha	2004

Soil sampling was conducted by taking multiple cores from each treatment to a depth of 7.5 cm for no till sites, and to 15 cm for cultivated sites, as described in the ARC protocol. The soil cores from each treatment were bulked among replicates, so that approximately 2 kg of soil from each treatment was collected. The samples were immediately frozen, and sent to ARC for bioassay analysis.

In 2003, and 2004, multiple crops were planted in the established recropping trials in 2 X 8 m strips. Visual evaluations for injury were taken shortly after crop emergence, at flowering, and prior to harvest. Yield was also taken for each crop.

Bioassay analysis by the ARC was conducted on the same crops that were planted into the field rotational cropping studies; canola, mustard, sugar beet, lentil, chickpea, canary seed and flax.

Results and Discussion

The information received from the Alberta Research Council included photographs of the bioassays and an accompanying letter with visual observations of the plant responses. If any injury was noted in the letter, then the plants were considered injured. For the field assessment, any visual injury rating of >15% recorded at any of the rating times was considered injury. This level of injury would likely result in a crop response inquiry from a producer. The results from the laboratory bioassay and the field bioassay were compared and categorized into three possible outcomes:

1. Corroboration - field and greenhouse bioassay results are the same.
2. False positive - injury occurs in the bioassay but is not observed in the field.
3. False negative - no injury is observed in the bioassay but is observed in the field.

A false positive result may represent lost opportunities for the grower or supplier, although it poses no risk to a grower or advisor. False negative may represent a risk or liability for the agronomist / supplier as the grower may suffer crop injury and/or economic loss if the grower proceeds.

The data is presented in two ways. The first includes all samples, while the second includes only those samples where injury was evident either in the field and/or the bioassay. Including all samples tends to skew the results, since there is a high degree of corroboration between the bioassay and field results when there is no injury in either test. In these soils, there is either no residue remaining, or the residue is below the sensitivity level of the crop being tested. Using only the data where injury was found ensures that only those soils with a detectable residue level are being evaluated.

AAFC Field Study

All samples: Field studies were compared with the canola and sugar beet bioassays. When all samples were included, 54% of the samples had some level of crop injury reported in either the field study or the bioassay, while 46% of the samples were symptom-free. Results from the field and the bioassay corroborated 78% of the time, while 17% were false positive, and 5% were false negative (Table 5).

Samples where injury evident in field and/or bioassay: The number of samples in which this occurred declined to 48. In this case, 63% of the samples corroborated, while 29% were false-positive, and 8% were false-negative (Table 6). If the sugar beet bioassay was used as a reference, then, 57% of the samples corroborated, while 39% were false-positive, and 4% were false-negative (Table 7). Sugar beet is more sensitive to Group 2 residues than canola; thus, it would be expected that the sugar beet bioassay would increase the number of false-positives and reduce the false negatives.

Table 5: Percent of samples in which the results from the ARC canola bioassay corresponded with results from canola field trials conducted at Scott, Melfort, and Vanscoy, SK. All samples taken are included.

Crop (Field)	Crop (Bioassay)	N-Value	Result	%
Canola	Canola	82	Corroboration	78
			False Positive	17
			False Negative	5

Table 6: Percent of samples in which the results from the ARC canola bioassay corresponded with results from canola field trials conducted at Scott, Melfort, and Vanscoy, SK. Only samples where injury was identified in the bioassay and/or the field are included.

Crop (Field)	Crop (Bioassay)	N-Value	Result	%
Canola	Canola	48	Corroboration	63
			False Positive	29
			False Negative	8

Table 7: Percent of samples in which the results from the ARC sugar beet bioassay corresponded with results from canola field trials conducted at Scott, Melfort, and Vanscoy, SK. Only samples where injury was identified in the bioassay and/or the field are included.

Crop (Field)	Crop (Bioassay)	N-Value	Result	%
Canola	Sugar beet	48	Corroboration	57
			False Positive	39
			False Negative	4

BASF Field Study

All Samples: The field study results for each crop were compared with the bioassay for the same crop for all samples that were analyzed (Table 8).

Canola: When all the analyzed samples were included, 34% of the samples had some level of injury found in the bioassay and/or the field, while 66% had no injury in either test. Results from the bioassay corroborated with field results 76% of the time, while 2% were false positive, and 22% were false negative.

Mustard: When all the analyzed samples were included, 40% of the samples had some level of injury found in the bioassay and/or the field, while 60% had no injury in either test. Results from the bioassay corroborated with field results 72% of the time, while 9% were false positive, and 19% were false negative.

Sugar beet: When all the analyzed samples were included, 83% of the samples had some level of injury found in the bioassay and/or the field, while 17% had no injury in either test. Results from the bioassay corroborated with field results 74% of the time, while 13% were false positive, and 13% were false negative.

Lentil: When all the analyzed samples were included, 10% of the samples had some level of injury found in the bioassay and/or the field, while 90% had no injury in either test. Results from the bioassay corroborated with field results 92% of the time, while 5% were false positive, and 3% were false negative.

Chickpea, Canary seed, and Flax: When all the analyzed samples were included, 0% of the samples had injury found in bioassay and/or the field, while 100% had no injury in either test. Results from the bioassay corroborated with field results 100% of the time.

Table 8: Percent of samples in which the results from the ARC bioassay corresponded with results from field trials. (All samples are included)

Crop (Field)	Crop (Bioassay)	N-Value	% Injured	% No Injury	Result	%
Canola	Canola	41	34	66	Corroboration	76
					False Postive	2
					False Negative	22
Mustard	Mustard	43	40	60	Corroboration	72
					False Postive	9
					False Negative	19
Sugar Beet	Sugar Beet	23	40	60	Corroboration	72
					False Postive	9
					False Negative	19
Lentil	Lentil	38	10	90	Corroboration	92
					False Postive	5
					False Negative	3
Chickpea, Flax and Canary Seed	Chickpea, Flax and Canary Seed	75	0	100	Corroboration	100
					False Postive	0
					False Negative	0

Samples where injury was evident in the field and/or the bioassay: The N-value for each crop declined substantially in this study due to the high number of samples not showing injury in either test (Table 9).

Canola: When only samples containing injury in either one or both tests were included, corroboration occurred 29% of the time, while 7% were false positive, and 64% were false negative.

Mustard: When only samples containing injury in either one or both tests were included, corroboration occurred 29% of the time, while 24% were false positive, and 47% were false negative.

Sugar beet: When only samples containing injury in either one or both tests were included, corroboration occurred 68% of the time, while 13% were false positive, and 13% were false negative.

Lentil: When only samples containing injury in either one or both tests were included, corroboration occurred 25% of the time, while 50% were false positive, and 25% were false negative.

Table 9: Percent of samples in which the results from the ARC bioassay corresponded with results from field trials. (Only samples where injury occurred in the bioassay and/or the field are used).

Crop (Field)	Crop (Bioassay	N-Value	Result	%
Canola	Canola	14	Corroboration	29
			False Postive	7
			False Negative	64
Mustard	Mustard	17	Corroboration	29
			False Postive	24
			False Negative	47
Sugar Beet	Sugar Beet	19	Corroboration	68
			False Postive	13
			False Negative	13
Lentil	Lentil	4	Corroboration	25
			False Postive	50
			False Negative	25

Using the Sugar beet bioassay to predict potential injury to canola in the field: In order to reduce the incidence of false negatives when predicting injury to canola using the bioassay, a more sensitive species is often used. Results from the sugar beet bioassay (most sensitive), and mustard bioassay (sensitivity < sugar beet but > canola) and their relationship with canola injury in the field are shown in Table 10. Only the samples that showed injury in the bioassay and/or field were used. When using sugar beet as an indicator for canola, the incidence of false positives (53%) greatly increased as was expected. Corroboration occurred 35% of the time, while false negatives still appeared 12% of the time.

Mustard used as an indicator for canola resulted in 35% corroboration, which is higher than the sugar beet bioassay. False positives occurred 20% of the time, and false negatives were found in 27% of the samples.

Using a more sensitive species to predict canola injury did not provide an accurate prediction of potential canola injury the field. False positives increased and false negatives still occurred.

Table 10: Percent of samples in which the results from the ARC bioassay corresponded with results from field trials when sugar beet and mustard were used as indicator species. (Only the samples where injury occurred in the bioassay and/or the field are used).

Crop (Field)	Crop (Bioassay)	N-Value	Result	%
Canola	Sugar Beet	17	Corroboration	35
			False Postive	53
			False Negative	12
Canola	Mustard	17	Corroboration	53
			False Postive	20
			False Negative	27

Differences between the Two Studies

The AAFC study resulted in a higher level of false positives (Tables 5 and 6) while the BASF study resulted in high levels of false negatives (Tables 8 and 9). Why the differences? There is no conclusive reason, but separating some of the AAFC data into soils from Scott and soils from other locations (Melfort, Vanscoy) provides some insight. Most of the false negatives in the AAFC study occurred at the other locations, although they were still much lower than the BASF data (Table 11). Scott is a highly responsive soil where imidazolinone carryover is common, and the corroboration between the bioassay and the field was acceptable. However, corroboration was much lower for the other locations. Another reason for the low corroboration in the BASF study may be that the samples from the replicates were combined and bioassay results were compared to the mean injury in the field. Observations on re-cropping trials have indicated significant variability in crop injury responses between replicates. Three replicates may have a treatment where there is no injury, while one replicate may have injury >80%. When the average is taken, the mean injury is greater than 15%. Combining samples from the four replicates (where three of them have no injury) may result in a dilution of the residue, which is not detected in the bioassay. However, this sampling protocol may be more representative of sampling conducted in a large field than the AAFC study. It should also be noted that the BASF study focussed exclusively on the imidazoline chemistry, while the AAFC study included other chemistries (Sundance, Everest, Frontline). It is possible that difference in results were contributed too, by the variation in behaviours of the chemistries in the bioassay and the field.

Table 11: Percent of samples in which the results from the ARC canola bioassay corresponded with results with field studies in the AAFC and BASF studies. The AAFC study has been separated into results from the highly responsive Scott soils and the other soils from Melfort and Vanscoy. (Only the samples where injury occurred in the bioassay and/or the field are used).

Study	Crop (Field)	Crop (Bioassay)	N-Value	Result	%
AAFC – Scott soils	Canola	Canola	31	Corroboration	71
				False Positive	26
				False Negative	3
AAFC – Other soils (Melfort, Vanscoy)	Canola	Canola	17	Corroboration	47
				False Positive	35
				False Negative	18
BASF	Canola	Canola	14	Corroboration	29
				False Positive	7
				False Negative	64

Conclusion

The bioassay provided a reasonable level of accuracy in predicting injury on the highly responsive Scott soils, and in predicting sugar beet (a very sensitive crop) injury in the field. However, the high incidence of false positives in the AAFC study and the high incidence of false negatives in the BASF study bring into question the ability of this tool to predict potential injury in the field. The high incidence of false negatives in the BASF study is of particular concern, as this carries a high degree of risk for the grower to incur crop and economic losses. Based on the data generated from these two studies, the bioassay should not be used to make non-label recommendations to growers, or too be used as a diagnostic tool. The potential for error in these studies has been minimized since the samples were taken from controlled trials with relatively small areas, with sampling conducted by experienced research staff. It is expected that sampling from a large, heterogeneous field landscape would increase the potential for error.

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