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Correlation of Chemical Analysis of Residual Levels of Aminocyclopyrachlor in Soil to Biological Responses of Alfalfa, Cotton, Soybean, and Sunflower

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Researchers, product registration personnel, and growers desire the ability to chemically detect residual amounts of herbicides in soil at concentrations below those necessary to cause phytotoxicity to sensitive nontarget or rotational crop plants. Alfalfa, cotton, soybean, and sunflower, crops sensitive to low concentrations of aminocyclopyrachlor in soil, were planted at field test sites approximately 1 yr after aminocyclopyrachlor methyl was applied. Soil samples were collected when rotational crops were planted and were analyzed for aminocyclopyrachlor by a method based on high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS), with a limit of detection (LOD) of 0.1 part per billion (ppb) (soil oven-dry weight basis). Loglogistic dose–response analysis correlated visual phytotoxic plant responses to residual concentrations of aminocyclopyrachlor in the soil. Concentrations of aminocyclopyrachlor estimated to cause 25% phytotoxicity to alfalfa, cotton, soybean, and sunflower were 5.4, 3.2, 2.0, and 6.2 ppb, respectively, 20 to 60 times greater than the LOD of the analytical method available for soil analysis. Results from these studies suggest this HPLC/MS/MS method of analysis can be used to indicate potential risk and severity of plant response for alfalfa, cotton, soybean, and sunflower, and for other plant species once dose–response curves for these additional species are established. This chemical assay may be particularly important if researchers desire to study the concentration, movement, and dissipation of aminocyclopyrachlor in soil or as part of a forensic investigation to better understand the cause of an unanticipated or undesirable plant response.

Nomenclature: Aminocyclopyrachlor; alfalfa, *Medicago sativa* L.; cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.; sunflower, *Helianthus annuus* L.

Key words: Aminocyclopyrachlor methyl, bioassay, herbicide, herbicide residues, mass spectrometry.

Investigadores, personal de registro de productos y agricultores, desean tener la habilidad para detectar químicamente cantidades residuales de herbicida en el suelo a concentraciones por debajo de aquellas necesarias para causar fitotoxicidad a las plantas sensibles que no se tratan de controlar o los cultivos de rotación. Cultivos sensibles a bajas concentraciones de aminocyclopyrachlor en el suelo, como la alfalfa, el algodón, la soya y el girasol, se sembraron en las parcelas en estudio, aproximadamente un año después que el aminocyclopyrachlor metil fuera aplicado. Se tomaron muestras de suelo cuando los cultivos de rotación se sembraron y se analizaron para detectar residuos de aminocyclopyrachlor con un método de cromatografía líquida de alta eficiencia/espectrometría de masa en tándem (HPLC/MS/MS), con un límite de detección de 0.1 partes por billón (ppb) (basado en el peso del suelo secado al horno). Un análisis log-logístico de dosis-respuesta correlacionó las respuestas visibles de fitotoxicidad observadas en las plantas a las concentraciones residuales de aminocyclopyrachlor en el suelo. Las concentraciones del herbicida que se estiman causaron 25% de fitotoxicidad a alfalfa, algodón, soya y girasol, fueron 5.4, 3.2, 2.0 y 6.2 ppb, respectivamente, y que son niveles de 20 a 60 veces mayores que el límite de detección del método analítico disponible para el análisis del suelo. Los resultados de estas investigaciones sugieren que el método de análisis HPLC/MS/MS puede usarse para indicar el riesgo potencial y la severidad de la respuesta de las plantas de alfalfa, algodón, soya y girasol. Para otras especies podría usarse luego que se establezcan curvas de dosis-respuesta para ellas. Esta prueba química podría ser particularmente importante si los investigadores desean estudiar la concentración, movimiento y disipación de aminocyclopyrachlor en el suelo, o como parte de una investigación forense para entender mejor la causa de una respuesta no anticipada o indeseable de unas plantas.

Farmers and applicators desire longer residual soil-active herbicides that degrade sufficiently to allow sensitive rotational crops or desirable plant species to be planted as part of a normal crop rotation. Aminocyclopyrachlor and aminocyclopyrachlor methyl are two recently discovered synthetic auxin-mimic herbicides with potential utility for residual broadleaf weed control in pastures, rangeland, and industrial rights of way, and for selected control of unwanted brush and trees in forestry (Claus et al. 2008). These pyrimidine carboxylic acid herbicides (Figure 1) have sufficient soil residual activity to provide at least several months of weed control. Pasture, rangeland, or forestry growers may decide to rotate out of their typical cropping patterns to plant other crops and must therefore be informed regarding when rotational crops can be planted safely into treated soil. This information is normally included in the rotational crop guidelines portion of the product label.

Occasionally, researchers, applicators, farmers, and production growers may desire to know residual levels of aminocyclopyrachlor in soil to better understand the physical

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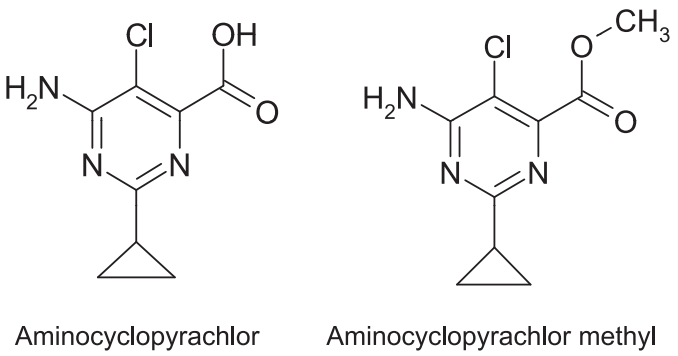

Figure 1. Chemical structures for aminocyclopyrachlor and aminocyclopyrachlor methyl.

properties of the active ingredient, to continue product development, or to collect information for forensic investigations. This information is normally not on the product label and must be generated as needed. The presence of residual levels of aminocyclopyrachlor in soil can be confirmed via a field bioassay or a chemical assay. A chemical assay offers several advantages over a plant bioassay (Clay 1993). A chemical assay can not only identify the chemical compound, but also accurately quantify residual concentrations of the active ingredient in soil; whereas, other herbicide active ingredients may interfere with a quantitative plant bioassay. A chemical assay can correlate the severity of plant response to a specific active ingredient; whereas, a bioassay measures plant response to aggregate stress factors present in soil. A chemical assay can usually be completed and results reported within a few days after soil sample collection; whereas, a plant bioassay may require considerable time to observe a specific plant response. A chemical assay can successfully measure active ingredient present within a wide range of concentrations; whereas, a bioassay may be quantitatively calibrated to only a narrow range of concentrations of active ingredient. A chemical assay also has several disadvantages (Eberle and Gerber 1976). Researchers may incorrectly assume that all residues (free and bound) are extracted from the sample. Chemical assays often require specialized analytical equipment and can be expensive. Samples may require extensive preparation and clean-up before a successful analysis is completed. In addition, the amount of residue extracted chemically may differ from the amount of residue biologically available to cause phytotoxic responses to bioassay species.

Two necessary and often challenging requirements for a chemical assay are correlating extractable herbicide levels with bioassay response and detecting residual concentrations of herbicide in soil that are at or below those necessary to cause phytotoxic responses in sensitive plant species. Nanita et al. (2009) recently reported a chemical assay method for detecting and quantifying aminocyclopyrachlor and aminocyclopyrachlor methyl at parts per billion (ppb) levels in soil. The method is based on detection with high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS). The objectives of this research were to determine if HPLC/MS/MS can detect and/or quantify aminocyclopyrachlor and aminocyclopyrachlor methyl at concentrations below phytotoxic amounts for alfalfa, cotton, soybean, and sunflower and to correlate the response of plants in a soil bioassay with detected amounts of these herbicides in a field study.

Materials and Methods

Greenhouse and Field Bioassays. Twenty-two crop species were grown under greenhouse conditions to select appropriate species for field trials. Seeds were planted into 10-cm pots containing a pH 6.5 Tama silty clay loam soil (Typic Argiudoll) with 2.9% organic matter (CEC = 19.2 meq 100 g^{-1}). Each pot contained a single plant species. One pot of each species (2 to 20 seeds per pot, depending on species) was planted for each herbicide treatment. Aminocyclopyrachlor methyl, formulated as a 25% wettable powder, was suspended in water and applied pre-emergence using a belt sprayer calibrated to spray 280 L ha^{-1} at 262 kPa. Herbicide application rates were 0, 0.25, 1, 4, 16, and 64 g ai ha^{-1}. Plants were grown in a greenhouse balanced with supplemental lighting (irradiance = 700 W m^{-2}) to maintain a 16-h photoperiod and set at 25 C daytime and 19 C nighttime temperatures. Plant responses were recorded on a 0 to 100% visual response scale on which 0 is no visible plant injury and 100 is plant death. Evaluations were made at approximately 21 d after treatment. This experiment was replicated four times.

The amount of herbicide to produce a 25% phytotoxic response (GR_{25}) was estimated with SAS using Proc Probit, version 9.[1]

For the field bioassay studies, formulated aminocyclopyrachlor methyl was applied at 0, 25, 50, 75, 150, and 300 g ai ha^{-1} to bare-ground plots in four locations in the United States (Table 1). All plots were maintained weed-free with glyphosate until rotational crops were planted. No herbicidal effects from glyphosate on planted rotational crops were observed in control plots, therefore ruling out potential biological interference in the study. Crop response was estimated visually using a 0 to 100% scale where 0 is no visible plant injury and 100 is plant death. Responses were recorded at approximately 1 mo after the crop was planted. Crop responses were also recorded at approximately 2 or 3 mo after planting at the Butlerville, IN, and the two Rochelle, IL,

Table 1. Soil characteristics of field test locations.

Location	Soil series	Texture	Soil type	pH	Organic matter (%)	CEC
Butlerville, IN	Rossmoyne	Silt loam	Aquic Fragiudalf	6.4	2.2	10.2
Rochelle, IL	Jasper	Loam	Typic Argiudoll	5.0	2.8	11.7
Rochelle, IL	La Hogue	Clay loam	Aquic Argiudoll	6.3	5.1	26.4
Newark, DE	Keyport	Silt loam	Aquic Hapludult	6.8	2.8	14.3

locations. Response ratings for these later evaluation times were lower or equal to ratings collected at the 1-mo evaluation dates (data not shown). Chemical analysis values were compared with crop bioassay responses at 1 mo after planting to establish relationships between residual aminocyclopyrachlor in soil to field crop response. There were three replicates of each treatment at each location.

Soil profiles were not disturbed at the Butlerville, IN, and the two Rochelle, IL, locations when crops were planted no-till into treated plot areas. At the two Rochelle, IL, locations, Pioneer '92M91' soybean[2] was planted 347 d after herbicide application. At the Butlerville, IN, location, Pioneer '94M30' soybean[2] and Pioneer '54H91' alfalfa[2] were planted 314 d after herbicide application. Soil samples (0 to 15 cm deep) were collected within a few days of planting rotational crops and were shipped overnight to the DuPont Stine-Haskell Research Center for cold storage at 4 C until chemical analysis was complete.

At the Newark, DE location, the top 3 cm of soil was tilled immediately before soybean[2] (Pioneer '92M91'), cotton[3] ('DP494 RR'), and sunflower[4] ('Hunters Select') were planted. Crops were planted 35, 128, 416, and 498 d after field plots were treated with aminocyclopyrachlor methyl. Soil samples (0 to 15 cm deep) were collected at 1 d after treatment to measure the initial amount of active ingredient applied to the soil and at 35, 128, 416, and 498 d after herbicide application to correlate chemical assay results with field crop response. Soil samples were stored at 4 C until chemical analysis was complete.

Chemical Analysis of Soil Samples. The procedure to measure concentrations of aminocyclopyrachlor and aminocyclopyrachlor methyl was based on the analytical method developed by Nanita et al. (2009), with a dilution factor adjustment (i.e., final volume after solid-phase extraction [SPE] of 5.0 ml instead of 15.0 ml as described below) to allow accurate quantitation of lower levels of active ingredients. The modification lowered the limit of quantitation (LOQ) from 1.0 ppb (ng g^{-1}) to 0.3 ppb (soil oven-dry weight basis), while the limit of detection (LOD) was approximately the same as previously reported, i.e., 0.1 ppb. The LOD is defined as the concentration of aminocyclopyrachlor estimated to produce a detector response approximately three times greater than the signal noise. Residues of aminocyclopyrachlor and aminocyclopyrachlor methyl were extracted from 10-g soil samples twice with 25 ml of acetonitrile (ACN)/0.15 M ammonium acetate (aqueous: aq) (70 : 30 v/v), and once with 25 ml of ACN/0.2% formic acid (aq) (80 : 20 v/v) by shaking at high speed on a wrist action shaker. Aliquots (5.0 ml) of the extracts were taken and evaporated to 1 ml using a nitrogen evaporator with the water bath temperature set at 40 C, and then diluted with 0.2% formic acid (aq) to 6 ml. The samples were loaded into an Oasis® MCX SPE cartridge[5] where the analytes were retained. Each cartridge was washed with 10 ml of methanol, and the analytes were eluted with 15.0 ml of 50 mM ammonium hydroxide in methanol into tubes containing 1.0 ml of 0.2% formic acid (aq). Samples were evaporated under nitrogen gas flow to 1 ml using a nitrogen evaporator (water bath temperature = 40 C) and then diluted to 5.0 ml with 0.01% formic acid (aq). A portion of each purified extract was filtered through a 0.45-µm PTFE filter[6] and analyzed following the exact HPLC/MS/MS instrumental analysis conditions previously described (Nanita et al. 2009).

Both aminocyclopyrachlor methyl and aminocyclopyrachlor were chemically assayed in soil samples collected from all field locations. Residual levels for both herbicides were reported in ppb (soil oven-dry weight basis). Results were reported on a carboxylic acid equivalent basis (i.e., total aminocyclopyrachlor equivalents) and compared to crop bioassay responses at 1 mo after planting. Loglogistic dose–response curves were used to model field bioassay responses of plant species to $\log_{10}$(measured concentration of aminocyclopyrachlor in ppb in soil) using Version 6.12 SAS for Windows® with PROC GENMOD[7] according to the general equation:

$$\text{Logit}(\text{response}) = \text{slope}\left[\log_{10}(\text{dose})\right] + \text{intercept} \qquad [1]$$

where Logit is defined as ln[response/(100 − response)] (McCullagh and Nelder 1989). Inverse confidence limits (95%) for the rate/concentration estimated to inhibit growth by x% (GR_x) were calculated using Fieller's theorem (Finney 1971) from loglogistic dose response curves. To visually compare predicted and observed plant responses in dose–response graphs, dose–response curves were back-transformed and predicted plant responses were calculated according to the equation:

$$\text{Predicted response} = \left\{e^{intercept + \left[slope \times \log_{10}(dose)\right]}\right\} \Big/ \left\{1 + e^{intercept + \left[slope \times \log_{10}(dose)\right]}\right\} \qquad [2]$$

Results and Discussion

Selecting Bioassay Species for Comparison of Chemical Analysis to Field Rotational Crop Results. In greenhouse studies, grass crops showed greater tolerance to aminocyclopyrachlor methyl in soil than broadleaf crops (Table 2). This bimodal distribution of crop tolerance and epinastic responses of broadleaf leaves and stems was consistent with that of other synthetic auxin herbicides (Ross and Lembi 1985). GR_{25} values for the nine grass crops tested were similar to greater than 64 g ai ha^{-1}, the highest rate applied in this greenhouse study. GR_{25} values for the 13 broadleaf crops ranged from 0.2 to 6.8 g ai ha^{-1}. Alfalfa, cotton, soybean, and sunflower were chosen for field rotational crop studies because these crops are sensitive to low levels of aminocyclopyrachlor in soil and seedling crop growth is uniform, thus reducing variation among plants for visual evaluations. Kidney bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.), and sugar beet (*Beta vulgaris* L.) appeared to be most sensitive to aminocyclopyrachlor methyl in soil. However, these crops were not chosen for chemical assay/field bioassay comparison studies because in these greenhouse studies, they exhibited reduced uniformity of early seedling growth and also expressed occasional spurious epinastic responses that could not be directly correlated with the amount of aminocyclopyrachlor methyl in soil.

Table 2. Rates of aminocyclopyrachlor methyl (g ai ha^{-1}) calculated to inhibit growth 25% (GR_{25}) when applied pre-emergence to crop species growing in a 2.9% organic matter silty clay loam soil in the greenhouse.

Crop	GR_{25}[a] (g ai ha^{-1})	95% Inverse Confidence Limits
Dicot		
Alfalfa (*Medicago sativa* L. 'Gem')	2.2	(1.4–3.0)
Canola (*Brassica napus* L. var. *napus*)	2.5	(1.3–3.8)
Clover, white (*Trifolium repens* L. 'Ladino')	1.0	(0.3–2.0)
Cotton (*Gossypium hirsutum* L. 'DP50')	3.3	(1.4–5.6)
Cucumber (*Cucumis sativus* L. 'Bush Champion')	4.1	(2.5–5.9)
Kidney bean (*Phaseolus vulgaris* L. 'Green podded bush')	0.2	(0.1–0.4)
Lentil (*Lens culinaris* Medik.)	0.6	(0.3–1.0)
Pea (*Pisum sativum* L. 'Laxtons progress 9')	1.7	(0.6–2.9)
Safflower (*Carthamus tinctorius* L.)	1.3	(0.4–2.6)
Soybean [*Glycine max* (L.) Merr. 'P94B53']	2.2	(1.1–3.6)
Sugar beet (*Beta vulgaris* L. 'Phoenix')	1.1	(0.2–2.3)
Sunflower (*Helianthus annuus* L. 'Peredovik')	6.8	(3.6–10)
Tomato (*Solanum lycopersicum* L. var. *lycopersicum* 'Marglobe')	2.3	(1.4–3.2)
Monocot		
Barley, spring (*Hordeum vulgare* L. 'Harrington')	> 64	n/a[b]
Bermudagrass [*Cynodon dactylon* (L.) Pers. 'Blackjack']	> 64	n/a
Bluegrass, Kentucky (*Poa pratensis* L.)	56	(28 to > 64)
Brome, smooth (*Bromus inermis* Leyss.)	> 64	n/a
Corn (*Zea mays* L. '33G26')	> 64	n/a
Fescue, tall (*Festuca* L. 'Sahara')	> 64	n/a
Oat, spring (*Avena sativa* L. 'Monida')	> 64	n/a
Rye, perennial (*Lolium perenne* L. spp. *perene* 'Trinity')	> 64	n/a
Wheat, winter (*Triticum aestivum* L. 'Stephens')	> 64	n/a

[a] GR_{25} is the rate estimated to inhibit growth by 25%. The highest rate tested was 64 g ai ha^{-1}.

[b] Abbreviation: n/a, not applicable.

Transformation of Aminocyclopyrachlor Methyl to Aminocyclopyrachlor. Chemical analysis of soil samples collected 1 d after herbicide application at Newark, DE, showed aminocyclopyrachlor methyl rapidly hydrolyzed to the acid form, aminocyclopyrachlor (Table 3). Quantifiable amounts of aminocyclopyrachlor methyl were detected only in field plots treated at the highest herbicide application rate of 300 g ai ha^{-1}. All soil samples from all field locations were analyzed for both aminocyclopyrachlor and aminocyclopyrachlor methyl. Aminocyclopyrachlor methyl was not detected in these soil samples and, if any active ingredient was present, it existed as aminocyclopyrachlor. This observation was consistent with the Newark, DE, results and the known rapid ester hydrolysis of aminocyclopyrachlor methyl to aminocyclopyrachlor (Turner et al. 2008). In addition, all samples were screened for a known soil degradation product of aminocyclopyrachlor, which is expected to be present in closed systems (i.e., soil samples) if active ingredient breakdown occurred during storage or sample preparation (Nanita et al. 2009). This compound was not detected, suggesting that sample storage at 4 C and sample handling procedures were satisfactory throughout the study. Subsequent correlations of field rotational crop responses were based solely on the amount of aminocyclopyrachlor measured in soil.

Table 3. Chemical analysis of soil samples (0 to 15 cm deep) collected 1 d after application of aminocyclopyrachlor methyl at Newark, DE.

Amount of aminocyclopyrachlor methyl applied (g ai ha^{-1})	Measured concentration of aminocyclopyrachlor (ppb)[a]	Measured concentration of aminocyclopyrachlor methyl (ppb)[a]
0	< 0.1[b]	< 0.1[b]
25	11.9	< 0.1
50	9.9	< 0.1
75	9.4	< 0.1
150	38.5	< 0.1
300	137.0	0.6

[a] Abbreviation: ppb, parts per billion.

[b] Limit of detection is 0.1 ppb on an oven-dry soil weight basis.

Chemical Assay Correlations of Field Rotational Crop Responses to Aminocyclopyrachlor. Field bioassay responses of cotton, soybean, and sunflower varied from no visual phytotoxicity to plant death with all three species exhibiting sigmoid dose–response curves to measured amounts of aminocyclopyrachlor in soil (Figure 2). For these three species, a minimum of 24 soil samples were collected with measured concentrations of aminocyclopyrachlor ranging from not detected to 63 ppb on a soil oven-dry weight basis. Concentrations of aminocyclopyrachlor in soil estimated to produce 25% phytotoxic response (GR_{25}) based on visual evaluations were 3.2 (2.2 to 4.0), 2.0 (1.2 to 2.7), and 6.2 (3.8 to 8.0) ppb for cotton, soybean, and sunflower, respectively (values are means and ranges for 95% inverse confidence intervals). Equations for dose–response curves are presented in Table 4. Based on visual observations, four data points in Figure 2 appear to be outliers. Estimates of GR_{25} values differed by 10% or less if these outliers were removed from

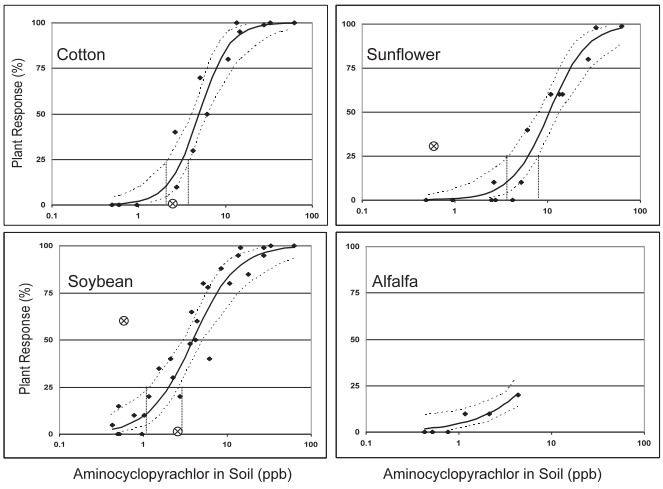

Figure 2. Observed (■) and predicted (—) responses (loglogistic dose response analysis) of cotton, soybean, sunflower, and alfalfa to measured concentrations of aminocyclopyrachlor in soil. Vertical dashed lines represent 95% confidence intervals for concentrations of aminocyclopyrachlor to produce 25% crop phytotoxicity. Data points for apparent outliers are circled. Cotton, soybean, sunflower, $P < 0.0001$; alfalfa, $P = 0.00015$ (--- 95% inverse confidence limits).

Table 4. Responses of alfalfa, cotton, soybean, and sunflower to measured concentrations of aminocyclopyrachlor in soil.

Rotational crop[a]	No. of samples	Test location(s)	GR_{25}[b] (ppb)[c]	Dose–response equation (Y)
All field data				
Alfalfa	6	Butlerville, IN	5.4 (3.7–27)[d]	$Y = [e^{-3.0968 + [2.72 \times \log10(dose)]}]/[1 + e^{-3.097 + [2.72 \times \log10(dose)]}]$
Cotton	24	Newark, DE	3.2 (2.2–4.0)	$Y = [e^{-4.0152 + [5.72 \times \log10(dose)]}]/[1 + e^{-4.0152 + [5.72 \times \log10(dose)]}]$
Soybean	42	All four field locations	2.0 (1.2–2.7)	$Y = [e^{-2.2823 + [3.83 \times \log10(dose)]}]/[1 + e^{-2.2823 + [3.83 \times \log10(dose)]}]$
Sunflower	24	Newark, DE	6.2 (3.8–8.0)	$Y = [e^{-4.9303 + [4.82 \times \log10(dose)]}]/[1 + e^{-4.9303 + [4.82 \times \log10(dose)]}]$
Apparent outliers removed				
Alfalfa	6	Butlerville, IN	5.4 (3.7–27)	$Y = [e^{-3.0968 + [2.72 \times \log10(dose)]}]/[1 + e^{-3.097 + [2.72 \times \log10(dose)]}]$
Cotton	23	Newark, DE	2.9 (1.9–3.7)	$Y = [e^{-3.4823 + [5.07 \times \log10(dose)]}]/[1 + e^{-3.4823 + [5.07 \times \log10(dose)]}]$
Soybean	39	All four field locations	1.8 (1.3–2.2)	$Y = [e^{-1.9806 + [3.62 \times \log10(dose)]}]/[1 + e^{-1.9806 + [3.62 \times \log10(dose)]}]$
Sunflower	23	Newark, DE	6.3 (4.7–7.6)	$Y = [e^{-4.9881 + [4.88 \times \log10(dose)]}]/[1 + e^{-4.9881 + [4.88 \times \log10(dose)]}]$

[a] Statistical significance: cotton, soybean, and sunflower, $P < 0.0001$; alfalfa, $P = 0.00015$.

[b] GR_{25} is the estimated dose to inhibit plant growth 25%.

[c] Abbreviation: ppb, parts per billion.

[d] 95% confidence interval.

the analysis. A threshold of 25% response was chosen as the upper limit generally acceptable for crop tolerance because this level of phytotoxicity approaches the lowest level of plant response that can be clearly associated with synthetic auxin herbicide mode of action and may be indicative of loss in crop yield (Andersen et al. 2004; Sciumbato et al. 2004). Fauci et al. (2002) studied pinto bean (*Phaseolus vulgaris* L.) response to the synthetic auxin herbicides, picloram and clopyralid, in compost. They reported ''no effect levels,'' based on visual observations of leaf formation of 1.3 to 2.5 ppb and 0.5 to 5 ppb of picloram and clopyralid, respectively.

Alfalfa response in field bioassays varied from 0 to 20% visual phytotoxicity (Figure 2). For these soil samples, measured concentrations of aminocyclopyrachlor varied from not detected to 4.5 ppb on a soil oven-dry weight basis. Statistical analysis of the dose–response data predicted 5.4 (3.7 to 27) ppb (mean and range of the 95% inverse confidence interval) of aminocyclopyrachlor in soil was sufficient to cause 25% crop phytotoxicity to alfalfa. The GR_{25} value for alfalfa should be viewed with a lower degree of confidence because the average response at the highest concentration of aminocyclopyrachlor measured was less than 25%.

The HPLC/MS/MS analytical method for aminocyclopyrachlor residues has a limit of detection (LOD) of 0.1 ppb in soil (Nanita et al. 2009). In these studies, soil concentrations of aminocyclopyrachlor necessary to produce GR_{25} response levels for cotton, soybean, sunflower, and alfalfa were 3.2, 2.0, 6.2, and 5.4 ppb, respectively, which corresponded to 32, 20, 62, and 54 times greater than the limit of detection (LOD) of the analytical method.

In greenhouse bioassay studies, kidney bean, lentil, and sugar beet were slightly more sensitive than alfalfa, cotton, soybean, and sunflower to the active herbicide (Table 2). In field studies, the amount of aminocyclopyrachlor required to produce 25% growth response in soybean was 20 times greater than the LOD of aminocyclopyrachlor, suggesting that this method of HPLC/MS/MS analysis should be capable of detecting concentrations of aminocyclopyrachlor in soil well below those necessary to produce 25% growth response for kidney bean, lentil, and sugar beet in commercial production environments.

A successful chemical assay for aminocyclopyrachlor in soil must first be able to detect the herbicide at or below concentrations necessary to injure sensitive plants. Soybean, cotton, sunflower, and alfalfa are four broadleaf crops sensitive to aminocyclopyrachlor. Based on results of these studies, the LOD of this HPLC/MS/MS method of analysis is 20 to 60 times lower than concentrations necessary to produce 25% phytotoxic responses for these sensitive plant species. In addition, a successful chemical assay should also be able to indicate the potential risk and severity of the phytotoxic plant response. This is particularly important if researchers and other interested parties desire to study the concentration, movement, and dissipation of aminocyclopyrachlor in soil in support of additional product development or as part of a forensic investigation to better understand the cause of an unanticipated or undesirable plant response. In these studies, a loglogistic model correlated phytotoxic responses of cotton, sunflower, and soybean to measured concentrations of aminocyclopyrachlor in soil, suggesting this HPLC/MS/MS method of analysis can be used to indicate the potential risk and severity of plant response for cotton, sunflower, and soybean, and for other plant species as dose–response curves for these additional species are established.

Sources of Materials

[1] Statistical Analytical Systems®, SAS user's guide, Statistics, 4th ed., 1990, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

[2] Soybean seed, Pioneer Hi-Bred International, Inc., 7100 NW 62nd Avenue, Johnston, IA 50131.

[3] Cotton seed, Delta and Pine Land Company, One Cotton Row, Scott, MS 38772.

[4] Sunflower seed, Southern States Cooperative, P.O. Box 459, Cloverdale, VA 24077.

[5] Oasis® MCX SPE cartridge, 500 mg, 6 cc, Waters Corporation, Milford, MA 01757.

[6] Syringe filter (25 mm) with 0.45-µm PTFE filter membrane, VWR Scientific Co., Bridgeport, NJ 08014.

[7] Statistical Analytical Systems®, SAS technical report, 1993, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

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