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Effect of postemergence applications of aminocyclopyrachlor, aminopyralid, 2,4-D, and dicamba on non–auxin resistant soybean

Research Article

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Abstract

Soybean [*Glycine max* (L.) Merr.] that lack resistance to auxin herbicides [i.e., not genetically modified for resistance] have well-documented responses to those particular herbicides, with yield loss being probable. When a soybean field is injured by auxin herbicides, regulatory authorities often collect a plant sample from that field. This research attempted to simulate soybean exposures due to accidental mixing of incorrect herbicides, tank contamination, or particle drift. This research examined whether analytical testing of herbicide residues on soybean to aminocyclopyrachlor (ACP), aminopyralid, 2,4-D, or dicamba would be related to the visual observations and yield responses from these herbicides. ACP and aminopyralid were applied to R1 soybean at 0.1, 1, and 10 g ae ha^{-1}; 2,4-D and dicamba were applied at 1, 10, and 100 g ae ha^{-1}. Visual evaluations and plant sample collections were undertaken at 1, 3, 7, 14, and 21 d after treatment (DAT), and yield was measured. The conservative limits of detection for the four herbicides in this project were 5, 10, 5, and 5 ng g^{-1} fresh weight of soybean for ACP, aminopyralid, 2,4-D, and dicamba, respectively. Many of the plant samples were non-detects, especially at lower application dosages. All herbicide concentrations rapidly declined soon after application, and many reached nondetectable limits by 14 DAT. All herbicide treatments caused soybean injury, although the response to 2,4-D was markedly lower than the responses to the other three herbicides. There was no apparent correlation between herbicide concentrations (which were declining over time) and the observed soybean injury (which was increasing over time or staying the same). This research indicated that plant samples should be collected as soon as possible after soybean exposure to auxin herbicides.

Introduction

Herbicides are commonly used in commercial farming in the United States and other areas of the world. Unfortunately, herbicides are sometimes misapplied to crops and may cause crop injury. Susceptible soybean [*Glycine max* (L.) Merr.] varieties can be injured from tank contamination or spray drift (Bovey and Meyer 1981; Solomon and Bradley 2014; Wax et al. 1969). While recent literature has focused primarily on vapor drift of dicamba (Bish et al. 2021), this report will focus on four different auxin herbicides with exposure routes being inadvertent mixing of the product, tank contamination due to incomplete clean out, or particle drift from an adjacent application area. These forms of soybean exposure to those herbicides are not uncommon in Tennessee (field observation). The four herbicides are 2,4-D (Anonymous 2024a), aminopyralid (Anonymous 2024b), aminocyclopyralid, ACP (Anonymous 2024c) and dicamba (Anonymous 2024d).

ACP is utilized on rights-of-way, pastures, industrial areas, and roadsides. The mechanism of action for ACP is that of a plant growth regulator, and it is highly mobile in the environment as well (Shaner 2014). Soybean are highly sensitive to ACP through direct leaf contact or soil contact through runoff situations (Solomon and Bradley 2014; Strachan et al. 2011). Aminopyralid is a pasture and non-crop herbicide (Anonymous 2024a). It is considered to be relatively nonvolatile (Senseman 2007). Both ACP and aminopyralid have label sections detailing the precautions for avoiding contamination of crops via various routes of exposure (Anonymous 2024a, 2024b). The normal use rates for ACP and aminopyralid are approximately 100 g ae ha^{-1} (Anonymous 2024a, 2024b).

The 2,4-D amine can be used in multiple crop species as well as in non-crop situations for the control of broadleaf weeds (Anonymous 2024c). More than 600 products containing 2,4-D are in commerce (Peterson 1967; Peterson et al. 2016; Song 2014). The 2,4-D amine was reported to be less injurious to soybean when applied at several growth stages compared with auxin herbicides (Solomon and Bradley 2014). The normal use rate for 2,4-D is 1,000 g ae ha^{-1}. Dicamba can be used as a burndown, preplant, preemergence, and/or postemergence application in dicamba-tolerant cotton (*Gossypium hirsutum* L.) and soybean for the control of broadleaf weeds. It is also used in pastures and turf weed control (Shaner 2014). Visual symptoms from dicamba applied at 0.01% of the labeled rate can occur on soybean (Jones et al. 2019; Scholtes et al. 2019). A common rate for dicamba is 560 g ae ha^{-1}.

The three potential scenarios examined in this research represent varying levels of herbicide dosages being applied to soybean. If an applicator chooses the wrong product (e.g., selecting the wrong herbicide container or possibly spraying the wrong field), the accidental doses can be relatively high, and this level of dosing was set at 10% of the potential use rate. The second potential source of contamination would be a small amount remaining in a spray tank from a previous application. While the amount would be highly variable, we selected a dosage of 1% of the normal application. Several dozen liters remaining in a large spray tank could easily result in a 1% mixture. Tank contamination can contribute to soybean response even if only trace amounts are present (Andersen et al. 2004; Scholtes et al. 2019). The final scenario was particle drift from an adjacent area, which was estimated to be 0.1% of the normal dosage. Grover et al. (1972) reported ~3% particle drift from a 2,4-D amine application. While all the scenarios could potentially have different concentrations of the different herbicides, this study investigated that a spread of two orders of magnitude would potentially represent at least some of the possible resulting outcomes.

Whenever herbicides are used, the dosage is an essential factor in how that herbicide works. The literature shows a wide range of potential use patterns for these four herbicides (Anonymous 2024a, 2024b, 2024c, 2024d). The rates chosen here represent a two-level factorial with two herbicides being used at a lower range, ACP and aminopyralid; and the other two herbicides, 2,4-D and dicamba, being used at a higher dosage range. While the dicamba dosage used in this research is based upon a rate greater than normally used in postemergence soybean production, a tank contamination issue or a drift pattern could easily result in this dosage. The formulations chosen were also those most commonly used in pastures and right-of-way applications to most accurately simulate an applicator causing particle drift onto adjacent soybeans.

The impact on soybean yield due to unexpected drift or contamination can vary. Visual effects can fluctuate based on the concentration of synthetic auxins present within the plant, the type of auxin herbicide, and the growth stage of the soybean plant (Al-Khatib and Peterson 1999; Solomon and Bradley 2014). Incidences of dicamba presence at early pod soybean growth stages directly correlate with reduced yield (Auch and Arnold 1978; Griffin et al. 2013; Jones et al. 2019; Wax et al. 1969).

Herbicide dosage clearly affects soybean response (Solomon and Bradley 2014; Zaccaro-Gruener et al. 2023). This research was designed to compare the effects of four auxin herbicides applied postemergence at three rates to simulate accidental exposure to non–auxin tolerant soybean. While there is a large amount of information on these herbicides' effects on soybean, there is no literature on a common field study examining all four herbicides and quantifying the herbicide residues immediately after application. The first objective of this study was to evaluate soybean response over time after herbicide application and to determine the effect on yield. The second objective was to quantify the specific herbicide present in the plant over time after application. The third objective was to assess the quantitative relationship between the measured herbicide concentration and the amount of soybean injury observed.

Materials and Methods

Field experiments were conducted in 2019 and 2020 at the East Tennessee Research and Education Center in Knoxville, TN (35.53°N, 83.57°W). The soil type was a Sequatchie loam, with sand/silt/clay of 36%, 40%, and 24%, respectively; pH of 6.3; and 1.9% organic matter. CEC of the soil was 7.1 mEq 100 g^{-1}. Glufosinate-tolerant soybean (Pioneer® P47A76L, Johnston, IA) were planted into conventionally tilled plots at 76-cm row spacing. Plots were maintained free of weeds by preemergence application of *S*-metolachlor and an early postemergence application of glufosinate. Postemergence maintenance herbicides were applied at least 14 d after treatment (DAT). A total of three field experiments were conducted. In 2019, the field study included evaluations of plant responses and leaf tissue samples collected for later analysis. There were two field studies in 2020: one that was the same as the 2019 field study (plant responses and sampling) and another that only measured plant responses to the herbicide treatments. Seeds for studies were planted on June 3, 2019, and May 14 and June 1, 2020.

Each individual experimental unit (plot) consisted of 6 rows that were 76 cm apart and 9 m in length. Only 6 m of the two center rows (labeled replicate A and B) of each plot received a treatment, which allowed 1.5 m on all sides of the treated area for a border. Visual evaluation of border rows did not show symptoms after treatment, so this amount of space between treated areas appeared to be adequate.

Four auxin herbicides were separately applied to soybean at the R1 stage of growth. This late growth stage was selected because this timing has been reported to produce the greatest soybean response (Solomon and Bradley 2014). The midsummer time interval is also a time when these products are being used in postemergence applications in pastures, rights-of-way, or agronomic crop settings. The four herbicides were ACP (Method®, 240 g ae L^{-1}, Bayer Crop Science, St Louis, MO, USA), aminopyralid (Milestone®, 240 g ae L^{-1}, Corteva Agriscience, Indianapolis, IN, USA), 2,4-D, (Weedar®, 480 g ae L^{-1}, Nufarm, Alsip, IL, USA), and dicamba (Clarity®, 480 g ae L^{-1}, BASF, Raleigh, NC, USA). The herbicides were applied on July 12, 2019, and July 6, 2020, to the experiments that had full sampling. The plant response-only study in 2020 had auxin herbicides applied on July 21, 2020. The herbicide applications were made during calm weather conditions to avoid off-target movement to adjacent plots. No in-season auxin-type herbicides were used in these plots or in any adjacent plots to avoid potential confusion from cross-contamination. This farm was also isolated from other soybean and cotton production areas, so no long-range dicamba drift occurred, which was a common occurrence in some areas of the United States in 2019 and 2020.

The four herbicides were divided into two general rate ranges, with ACP and aminopyralid having a prospective full use rate of 100 g ha^{-1}, and 2,4-D and dicamba having a possible full use rate of 1,000 g ha^{-1}. This full rate was not applied, but a factorial of 10%, 1%, and 0.1% of each herbicide was applied in separate treatments to soybean. Untreated checks were included in the design for comparison purposes. These three herbicide doses attempted to simulate possible tank-mixing errors (10%), spray-tank contamination issues (1%), or particle drift (0.1%).

Research was conducted using a factorial arrangement of treatments in a randomized complete block design with three replications in which each plot was an experimental unit. Untreated border rows were located between each treated area. Herbicides were applied to the designated area within the plot

Table 1. Parameters for LC/MS analytical methods for four auxin herbicides examined in field studies in Knoxville, TN

Compound	Analytical column	MS mode	Parent ions	Confirmation ions	Retention time	Limit of detection[a]	Recovery
			m/*z*		min	ppb	%
Aminocyclopyrachlor	Amino	Positive	214	196	1.8	0.5	57
				168			
Aminopyralid	Phenyl-hexyl	Positive	207	188.9	1.27	1	75
			206.8	160.9			
				133.9			
				161			
2,4-D	Phenyl-hexyl	Negative	219	160.9	4.6	0.5	98
				125			
Dicamba	Phenyl-hexyl	Negative	221	177	3.9	0.5	75
			219	175			
				145			

[a] Limit of detection on instrument stated; limit of quantitation in soybean leaves was 10 times this number.

using one pass with a handheld CO_2-pressurized backpack sprayer calibrated to spray 187 L ha^{-1} at 276 kPa using AIXR 8002 nozzles (TeeJet® Technologies, Wheaton, IL USA). The sprayer boom had four nozzles spaced 50 cm apart, and plots were sprayed in the midmorning after the dew on the soybean plants had dried. Filter papers placed inside the plot area before herbicide application were collected after treatment and later analyzed to provide an indication of the initial herbicide dosage applied to each plot.

Soybean were observed for visual symptoms at 3, 7, 14, and 21 DAT. A scale of 0 to 100 was used to assess crop response, with 0 being no symptoms and 100 being complete necrosis (Sciumbato et al. 2004). Plant response varied from minor to substantial, and included leaf cupping, leaf crinkling, leaf curling, galls, epinasty and complete necrosis (Al-Khatib and Peterson 1999; Andersen et al. 2004; Auch and Arnold 1978; Peterson 1967; Wax et al. 1969). Border rows for each plot were inspected for symptoms at respective sampling intervals to assess potential drift between plots.

Analytical Methods

Two randomly selected leaves (one full trifoliate leaf was the sample size) were collected from each plot at the same time interval as the visual observations (1, 3, 7, 14, and 21 DAT). Rainfall immediately after application precluded sample harvest at 1 DAT in 2019. The harvested leaves were placed in a plastic bags and immediately put into a cooler and then stored in a freezer (−20 C) until later analysis. Contamination of plant samples when conducting field studies from herbicide-treated plots is problematic (Riter et al. 2023). Care was taken to avoid cross-contamination between the different samples, including changing gloves after the collection of every subplot.

An experimental design consideration was the use of a single set of plots for both the herbicide concentration determinations and the soybean responses (visual evaluations and final soybean yield). If a large plant sample had been collected and removed from each plot at the various sampling intervals, the efficacy and yield data would have been compromised. The total amount of soybean material analyzed in each sample (average 2.3 g) was similar to the 3- to 5-g samples used by Zaccaro-Gruener et al. (2023). The soybean leaf samples were randomly selected and thus would be considered representative of the treated area, and each plot was sampled more than once at each interval.

Soybean leaf samples were homogenized using a mortar and pestle with liquid nitrogen poured onto the sample. Each trifoliate leaf (three leaflets) was composited into that sample with mean, minimum, and maximum fresh weights among the 698 samples being 2.27, 0.52, and 6.57 g (data not shown). Field replicates A and B were kept separate throughout lab procedures. After the sample had been homogenized, the contents were placed into a 50-ml conical tube. The samples were then placed in a freezer at −18 C. Before analysis, samples were thawed, and 30 ml of acetonitrile was added to the tubes, which were then shaken overnight (~16 h). ACP and aminopyralid samples were taken off the shaker the following morning and filtered. Dicamba and 2,4-D samples received 500 µl NaOH, and the resulting mixture equilibrated at room temperature (21 C) for ~30 min. HCL (500 µl) was added to each tube, mixture heated in 60 C water bath for 30 min, allowed to cool, and centrifuged. Luer-lock tip syringes (10 ml) with a 25-mm syringe filter were used to filter extracts into 2-ml high-performance liquid chromatography vials with corresponding labels for later analysis.

Lab analysis used an Agilent 1260 liquid chromatograph coupled with an Agilent 6470 mass spectrometer (Santa Clara, CA). All separations used a gradient of water + 0.1% formic acid and acetonitrile + 0.1% formic acid (all reagents MS grade). Details of the analyses, including stationary phase, parent ions, confirmatory ions, and other details, are in Table 1. Soybean leaves from non-treated plots were analyzed to verify that no auxin herbicides were present (data not shown), and leaves were also fortified with known amounts of each auxin herbicide and the recovery determined. Recoveries from these fortified samples ranged from 57% to 98% (Table 1). Concentration data presented have been corrected for recoveries. The limit of quantitation was set at a signal-to-noise ratio of 10 to 1 and was 5, 10, 5, and 5 ng g^{-1} fresh weight for ACP, aminopyralid, 2,4-D, and dicamba, respectively. One challenge of laboratory methods is to develop and validate analytical methods that are accurate, precise, and sensitive. To that end, the MS operating mode, the parent ion, and the confirmatory ions listed in Table 1 can provide a starting point for method development for analysts.

Statistical analysis was conducted using the PROC GLIMMIX feature within SAS (SAS v. 9.4, SAS Institute, Cary, NC, USA). There were no differences or interactions among the three studies, so the data for injury and yield were pooled. Fixed effects were herbicide and rate. Study, replication and any interactions were considered random effects in the statistical model. Mean separation for individual treatment differences was performed using Fisher's protected LSD at $P \leq 0.05$.

The herbicide concentration data from the highest dosage of each chemical were empirically fit to the equation:

Table 2. Temperature (average of maximum and minimum for that day) and rainfall for field studies from which plant responses and plant samples were collected, referenced to the day of auxin herbicide application

	2019		2020	
DAT[a]	Avg. temp.	Rainfall	Avg. temp.	rainfall
	C	mm	C	mm
−5	26.7	0	23.33	0
−4	25.8	0	23.89	1
−3	26.4	0	25.83	0
−2	27.5	0	26.11	0
−1	27.2	2	26.11	0
0	25.0	2	25.56	0
1	25.6	27	28.33	0
2	25.8	82	24.72	0
3	25.6	0	25.83	0
4	26.1	0	26.11	0
5	26.9	3	27.78	15
6	24.7	8	26.11	0
7	25.6	0	24.44	0
8	27.5	0	26.11	0
9	27.2	0	26.11	0
10	26.1	3	27.22	0
11	23.3	113	29.44	0
12	19.2	0	29.44	0
13	20.0	0	29.72	0
14	21.7	0	27.22	0
15	22.5	0	29.72	25
16	23.6	0	28.61	0
17	24.4	0	29.17	3
18	23.3	0	25.83	10
19	23.3	0	25.83	12
20	24.2	0	26.67	0
21	25.3	7	26.94	3

[a] DAT, days after treatment.

$$f = a * e^{(-b*x)} \qquad [1]$$

where a is the y intercept and represents the hypothetical initial herbicide concentration, and b represents the first-order rate constant (k) empirically fitting the data of herbicide decline over time. This two-parameter, single exponential decay model fit most of the data curves relatively well, with some exceptions. Herbicide half-lives were calculated by the relationship:

$$\text{Half-life} = 0.693/k \qquad [2]$$

The soybean injury data were regressed using a simple linear model with the equation:

$$y = mx + b \qquad [3]$$

where m represents the slope of the line, and b represents the y intercept. The x axis was the herbicide concentration, and the y axis was the observed soybean injury at each evaluation sampling interval. The r^2 from this regression was used to measure the correlation between these two factors. The regression analyses were performed using SigmaPlot v. 14.0 (Grafiti LLC, Palo Alto, CA).

Results and Discussion

Environmental conditions for the field studies were typical for the growing region in the U.S. Midsouth, with average temperatures between 22 and 27 C each day and adequate soil moisture to allow for good soybean growth (Table 2). Rainfall immediately after herbicide application in 2019 precluded sampling until 3 DAT.

All herbicides at all rates caused some level of soybean injury (Table 3). All herbicides had a pronounced rate response, with soybean injury being greater at the higher rates. This observation was expected, given the large differences in our factorial arrangement of treatments. In general, soybean injury from 3 to 21 DAT increased with ACP, aminopyralid, and dicamba, whereas injury was static or decreased with 2,4-D. Final soybean yield was inversely related to observed injury, with lower observed yields in plots with greater herbicide injury.

When comparing ACP and aminopyralid at the same rates, aminopyralid normally caused more soybean injury and lower yields (Table 3). Dicamba caused the greatest injury of all treatments, and this was expected, because it is well established as injurious to soybean (Jones et al. 2019). The 2,4-D treatments caused much less soybean response than dicamba at the same rates. This is consistent with other previously established reports (Grover et al. 1972; Wax et al. 1969).

Whenever experiments are done in small plots, it is challenging to have accurate and precise herbicide applications. The herbicide concentrations from the filter paper samples provided a simple method of estimating the dosage applied to each plot. Although a small data set (three per plot), the observed application dosages were consistent with the targeted application rates (Table 4). The relative proportion between the three different rates was also consistent and always changed by a factor of ~10, which was the intent of the study. While the small number of subsamples does not absolutely validate the dosage applied to each plot, it provides at least some evidence that the initial dosing was correct.

When lower herbicide rates are applied and subsequently sampled, the treated plants would be expected to have lower herbicide concentrations, and thus the level of sensitivity is very important in the interpretation of results. The limit of quantitation in this study was defined as a signal-to-noise ratio of 10 times on our instrument, when given the extraction efficiency, the limit of detections was 5, 10, 5, and 5 ng g^{-1} fresh weight for ACP, aminopyralid, 2,4-D, and dicamba, respectively. There were many non-detects in the herbicide analysis (Table 5). The lowest doses of all materials had the highest percentage of non-detects. Conversely, the highest doses usually had a small number of non-detects. Because no transformation of the concentration data was performed in a later correlation analysis, a value of zero was assigned for the non-detects. While the actual herbicide concentration value is unknown (because it is below the limit of detection), a value of zero would have little effect on the overall correlation, given the magnitude of the observed values that were measured (Table 6). This large number of non-detects is consistent with results reported by Zaccaro-Gruener et al. (2023).

Large deviations in herbicide concentrations were observed (Table 6). Possible explanatory factors include the wide range of initial application dosages, differential behavior within the soybean plants, sampling error, or perhaps loss mechanisms before each herbicide entering into the soybean plant (Table 6). Both ACP and aminopyralid had few to no detections at the lowest application dosage. Both 2,4-D and dicamba had non-detects in the later sampling intervals at the lower application rates. All herbicide concentrations decreased over time, which is consistent with Zaccaro-Gruener et al. (2023). One difference from the other three herbicides was that aminopyralid at 1 and 10 g ha^{-1} declined from 1 to 7 DAT and then increased in the later sampling intervals (Table 6). This trend was consistent across all replications and all

Table 3. Effect of auxin herbicides applied postemergence to soybean[a]

Herbicide[b]	Dose	3 DAT	7 DAT	14 DAT	21 DAT	Yield
	$g\ ha^{-1}$	soybean injury %				$kg\ ha^{-1}$
ACP	0.1	2.1 FE	8.2 G	12.8 F	11.7 H	4,140 AB
ACP	1	7.7 DC	14.3 F	26.1 E	28.9 F	3,250 C
ACP	10	38.2 C	44.3 C	50 C	57.8 C	583 E
Aminopyralid	0.1	1.6 FE	8.2 G	11.7 GF	13.9 H	3,960 AB
Aminopyralid	1	11.6 C	17 FE	28.3 E	37.2 E	2,520 D
Aminopyralid	10	49.9 A	57.6 B	70.6 B	73.3 B	182 EF
2,4-D	1	0 F	0 H	1.4 H	2.3 I	4,230 A
2,4-D	10	4.4 DE	5.9 G	7.2 GF	3.9 I	4,220 A
2,4-D	100	33.8 B	30.4 D	28.3 E	20 G	3,780 B
Dicamba	1	4 DE	17 FE	26.7 E	30 F	3,120 C
Dicamba	10	12.1 C	20.4 E	35 D	46.1 D	2,320 D
Dicamba	100	52.1 A	73.2 A	92.8 A	96.1 A	49 F
None, UTC	0					4,310 A
LSD (0.05)		4.2	5	4.98	5.4	403

[a]Visual evaluations are from 3 to 21 d after treatment (DAT), and soybean yield at end of season from three trials conducted in Knoxville, TN. Means within a column not followed by a common letter are significantly different ($P < 0.05$).
[b]ACP, aminocyclopyrachlor; UTC, untreated control.

Table 4. Herbicide application dosage based upon field samples collected at the time of herbicide application from field experiments in Knoxville, TN, in 2019 and 2020[a]

Herbicide[b]	Target rate	Mean ± SE	
	$g\ ha^{-1}$		
ACP	0.1	0.10	0.02
ACP	1	0.97	0.12
ACP	10	10.46	1.94
Aminopyralid	0.1	0.17	0.02
Aminopyralid	1	1.69	0.38
Aminopyralid	10	19.96	0.76
2,4-D	1	0.82	0.10
2,4-D	10	9.26	1.19
2,4-D	100	77.24	17.24
Dicamba	1	1.43	0.37
Dicamba	10	12.08	2.04
Dicamba	100	125.19	43.72

[a]Mean and SE of 6 replications, pooled over 2 yr.
[b]ACP, aminocyclopyrachlor.

Table 5. Herbicide results (percentage of non-detects) of four auxin herbicides applied to R1 soybean and subsequently sampled at 1 to 21 d after treatment[a]

Herbicide	Dose applied	2019	2020
	$g\ ha^{-1}$	%	
ACP	0.1	100	100
ACP	1	60	50
ACP	10	13	27
Aminopyralid	0.1	93	90
Aminopyralid	1	40	60
Aminopyralid	10	0	13
2,4-D	1	100	73
2,4-D	10	60	57
2,4-D	100	53	30
Dicamba	1	90	100
Dicamba	10	33	67
Dicamba	100	3	0

[a]Field study conducted in Knoxville, TN, in 2019 and 2020. Data presented as percent of total samples for that treatment, so 100% indicates all results for that treatment were less than the limit of detection. Limit of detection was 5, 10, 5, and 5 $ng\ g^{-1}$ fresh weight for aminocyclopyrachlor (ACP), aminopyralid, 2,4-D, and dicamba, respectively. Each value based upon 30 samples (5 sampling intervals, 3 field replications, 2 subsamples from each individual plot).

studies, so it would not easily be explained by sampling error. Perhaps this molecule is more likely to be sequestered in the sample plant part and thus available for later extraction and analysis.

Comparing 2,4-D and dicamba residue levels, both had similar initial concentrations at 1 DAT; but 2,4-D rapidly decreased to nondetectable levels at 21 DAT, while dicamba concentrations remained high throughout the sampling interval at the highest dosage. One difference between this research and the study by Zaccaro-Gruener et al. (2023) was that the Arkansas researchers used the respective herbicide-tolerant soybean for 2,4-D and dicamba, and those soybean have mechanisms to degrade the respective auxin herbicides; and thus those authors measured very low concentrations of 2,4-D and dicamba in their soybean. This differential herbicide metabolism would readily explain why we had higher concentrations of 2,4-D and dicamba detected at the later intervals.

The herbicide concentration data from the highest initial dose of each herbicide were pooled across year and all replications, and those data were regressed against time. The individual data points were used in the regression analysis (Figure 1), and the predicted regression line, mean ± standard error, half-life, and r^2 of each respective treatment are shown. The relative error in the first initial sampling is higher than in the other sampling intervals, which is consistent with most herbicide environmental samples (Mueller and Senseman 2015). All herbicides in our research followed a similar pattern: rapid decline and all half-lives less than 2 d, although 2,4-D's half-life was shorter than the other three based on a comparison of first-order rate constants and confidence intervals. While the fit to the data of the first-order regression line was not perfect (average of the four r^2 values was 0.56), the r^2 was sufficient to provide an indication of herbicide behavior in soybean after postemergence application.

Andersen et al. (2004) published a similar report examining dicamba and 2,4-D and soybean. The plant responses and yield results were largely similar to these current findings, although those authors treated an earlier-stage soybean. There was also a similar decline in both 2,4-D and dicamba concentrations over time. Anderson et al. (2004) suggested that plant samples should be collected as soon as possible after suspected herbicide exposure for accurate detection and quantification of said residue. While they

Table 6. Herbicide concentrations of four auxin herbicides applied to R1 soybean and subsequently sampled at 1 to 21 d after treatment (DAT)[a]

Herbicide[b]	Dose applied	1 DAT mean	1 DAT SE	3 DAT mean	3 DAT SE	7 DAT mean	7 DAT SE	14 DAT mean	14 DAT SE	21 DAT mean	21 DAT SE
	g ha^{-1}	ng herbicide g^{-1} fresh weight (ppbw)									
ACP	0.1	0.0		0.0		0.0		0.0		0.0	
ACP	1	63.8	42.4	9.4	15.8	34.8	60.1	8.7	14.0	1.6	5.6
ACP	10	1,084.8	844.8	318.2	250.5	266.6	291.7	26.1	31.7	6.7	11.0
AMP	0.1	0.0		3.1	7.3	0.0		0.0		9.2	17.2
AMP	1	40.1	32.9	12.6	16.2	2.4	8.3	8.6	12.8	39.3	16.3
AMP	10	751.4	425.3	299.3	119.5	80.1	53.2	33.9	18.6	37.5	18.5
2,4-D	1	32.1	14.9	1.9	4.5	0.0		0.0		0.0	
2,4-D	10	212.5	93.6	19.5	19.2	2.4	6.1	9.9	34.3	0.0	
2,4-D	100	3,384.0	1,562.7	462.1	507.1	163.7	240.7	13.5	27.9	0.0	
Dicamba	1	611.4	947.2	0.0		10.3	21.0	0.0		0.0	
Dicamba	10	260.6	202.7	86.1	277.6	278.3	439.2	35.5	85.0	4.8	14.8
Dicamba	100	4,511.8	3,197.7	1,639.8	1,225.8	718.5	310.4	907.4	851.0	947.7	1,450.7

[a] Field study conducted in Knoxville, TN, in 2019 and 2020. Each value based upon 12 samples (2 yr, 3 field replications, 2 subsamples from each individual plot). The 1 DAT samples not collected in 2019 due to weather constraints. Values less than the limit of detection were denoted as 0, and they were assigned an SE of zero.

[b] ACP, aminocyclopyrachlor; AMP, aminopyralid.

performed a number of correlation analyses on their data, they did not directly correlate herbicide concentration to observed injury at that time interval. They demonstrated a relationship between herbicide concentrations and final grain yield.

Griffin et al. (2013) reported 19% soybean injury from 1 g ha^{-1} dicamba, which is consistent with our results. They also reported soybean were 2.5 times more sensitive to dicamba response at flowering, which was the reason we chose to apply treatments at R1 in this study. Auch and Arnold (1978) also showed more dicamba effect at the R1 growth stage, and our findings are consistent with their report. Wax et al. (1969) reported more injury from dicamba compared with 2,4-D, even when dicamba doses were low. Their report also showed less effect from 2,4-D on soybean.

A specific objective of this project was to correlate the observed herbicide concentrations with the observed herbicide injury of the soybean plants at that specific time. This research question is salient, because interested parties would like to take a plant sample at a specific time and determine whether that herbicide was causing the symptomology and what the overall economic impacts may be. From a qualitative perspective, this is problematic, because the injury is increasing or remaining the same over the first 3 wk after herbicide exposure, but the apparent herbicide concentration is declining. In many real-world scenarios, the actual date of the initial herbicide exposure to the soybean is unknown. Still, in an attempt to show the relationship, the herbicide concentrations of each herbicide were pooled across all factors and regressed against the observed visual injury (Figure 2). While all of the predicted lines had a positive slope, the actual fit to the data was poor, with no clear relationship between herbicide concentration and observed injury ($r^2 < 0.3$).

Because there is often a lag time between the observation of symptoms and the possible collection of plant samples, a second correlation was conducted. The data were censored, with only those observations from 14 to 21 DAT considered, and a similar regression analysis was conducted (Figure 3). While some r^2 values improved (possibly because the range of herbicide concentrations is substantially smaller), there still was no apparent relationship between herbicide concentration and observed visual injury.

The three objectives of this project were to examine soybean response from four auxin herbicides, quantify those herbicides in the treated plants, and finally to correlate these two observations. Soybean response was consistent with previous reports; ACP, aminopyralid, and dicamba caused the most soybean injury and

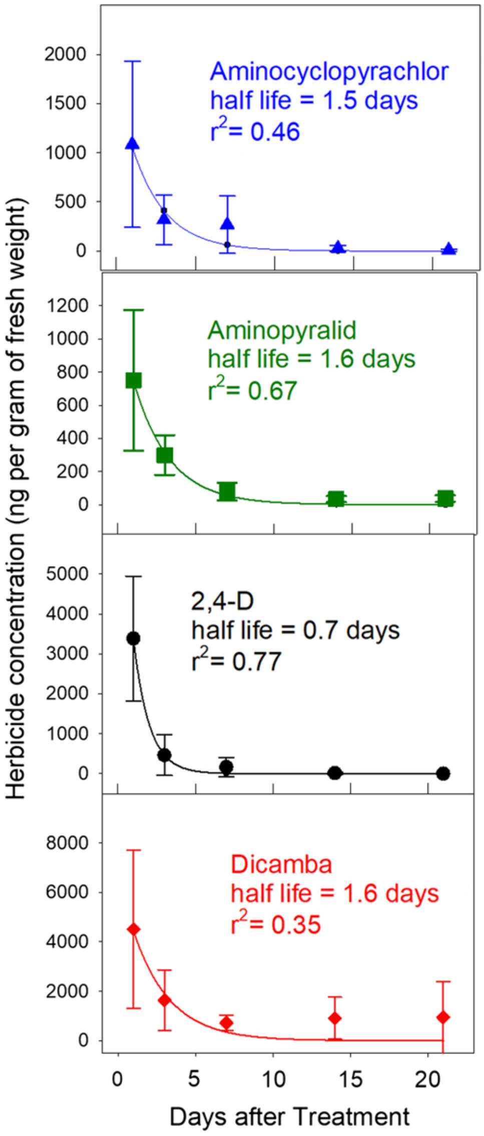

Figure 1. Herbicide concentrations from 1 to 21 d after treatment from plots treated with 10 g ha^{-1} of aminocyclopyrachlor and aminopyralid and 100 g ha^{-1} of 2,4-D and dicamba from field research in Knoxville, TN, in 2019 and 2020. Half-life and r^2 values based upon first-order regression of the raw data from each individual graph. Data points shown in the figure represent the mean ± SE bars.

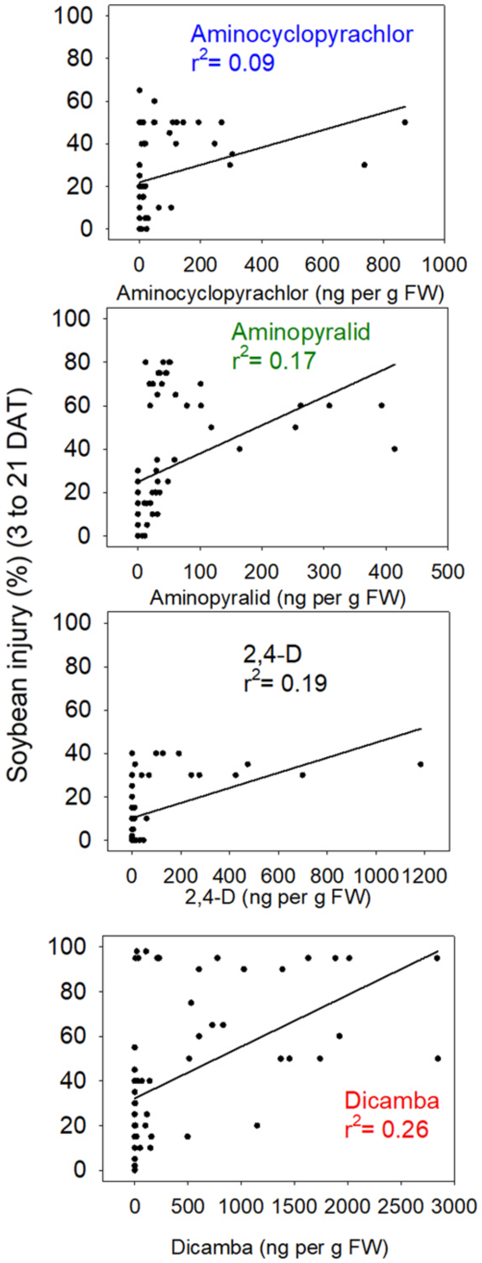

Figure 2. Relationship between measured herbicide concentration and observed soybean injury at 3 to 21 d after treatment from field plots near Knoxville, TN, treated with four auxin herbicides at the R1 stage in 2019 and 2020. Non-detects for herbicide concentrations were valued at zero for the purpose of this correlation analysis. FW, fresh weight.

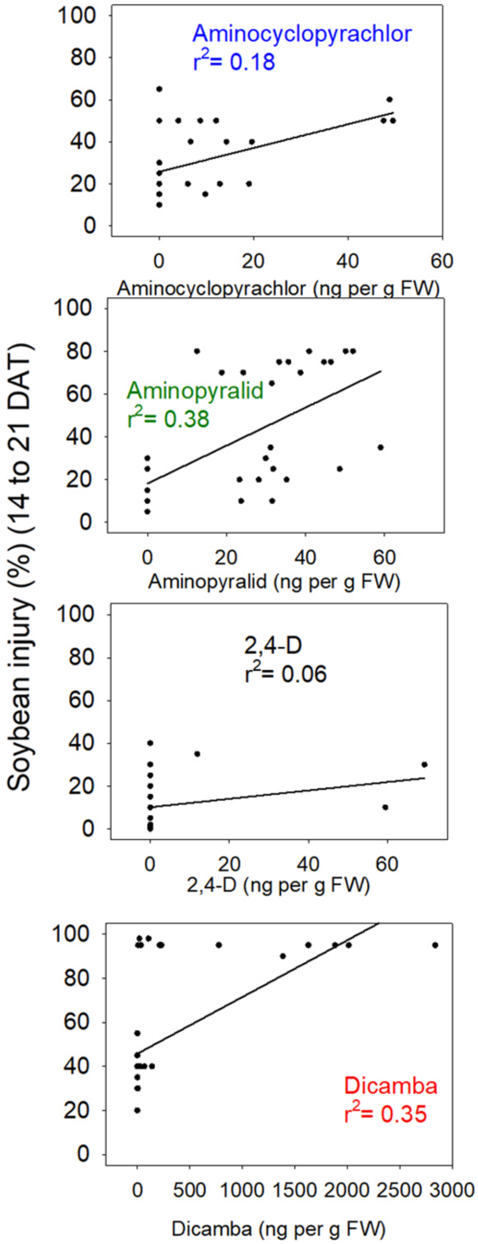

Figure 3. Relationship between measured herbicide concentration and observed soybean injury at 14 to 21 d after treatment from field plots near Knoxville, TN, treated with four auxin herbicides at the R1 stage in 2019 and 2020. Non-detects for herbicide concentrations were valued at zero for the purpose of this correlation analysis. FW, fresh weight.

reduced yield, while 2,4-D produced less injury and less yield reduction. All herbicide concentrations rapidly declined over time, often to nondetectable levels. No correlation was readily apparent between the observed herbicide concentration and soybean injury. This last observation illustrates the conundrum of wanting to get a plant sample to “prove” a crop injury occurrence, where in reality, the chemical analysis may not be as sensitive as the observed plant response. There may be value in a qualitative concentration assessment, meaning that if a given herbicide were present, that would lend credence to the observed injury, rather than the absolute value of the measured concentration. Nevertheless, this project indicates the urgency of collecting plant samples as soon as possible suspected exposure of soybean to auxin herbicides.

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