Relative Soybean Response to Aminocyclopyrachlor, 2,4-D, Dicamba, and Aminopyralid

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Abstract

This research compared the visual symptomology of four auxin herbicides applied at three rates to simulate tank contamination on non-auxin tolerant soybeans. The herbicides examined were aminocyclopyrachlor (ACP), 2,4-D, dicamba, and aminopyralid. The standard labeled rates (SLR) used to base the application rates were 0.12 kg ae ha^{-1} for ACP and aminopyralid while dicamba and 2,4-D used 1.12 kg ae ha^{-1}. These four herbicides exhibit similar symptoms on soybeans which is problematic when diagnosing soybean response. One goal of this project was to obtain high-quality images of the four herbicide's effect on soybeans, with the hope of being able to discern the herbicides effects on soybeans solely from visual symptoms. Each herbicide was applied at three different rates: 0.1X, 0.01X, and 0.001X of the SLR. The application was made at the R1 soybean stage. A randomized complete block design with three replications was utilized. Border rows for each plot were inspected for injury to assess potential drift between plots. The plots were visually evaluated for herbicide response at 3, 7, 14, and 21 days after treatment (DAT). Two trifoliate leaves were collected during each evaluation for future chemical analysis (data not shown). At 21 DAT, all 0.1X treatments had greater than 20% crop injury. The 0.001X rate for each herbicide exhibited different symptomology when compared to 0.1X rate. Leaf cupping and galls were more prominent on soybeans at lower herbicide dosages. Increasing the herbicide dosage changed symptomology from leaf cupping and galls to necrosis, leaf curling, and epinasty. There were no distinct visual differences between herbicides. Soybean yield and herbicide dosage were inversely related, and each herbicide affected soybeans differently. Treatments containing 2,4-D caused less soybean response than the other auxin herbicides. Dicamba at 0.001X reduced soybean yield 19%. Aminopyralid and ACP at 0.01X reduced soybean yields 43% and 16%, respectively. The general ranking of the relative soybean

injury caused by the auxin herbicides in this research was 2,4-D < ACP < aminopyralid < dicamba.

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CHAPTER 1

Post-emergence Auxin Applications on Non-auxin Resistant Soybeans

Post-emergence Auxin Applications on Non-auxin Resistant Soybeans

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Abstract

Auxin herbicides have been a key component to weed management in cropping systems and non-crop settings. Dicamba, 2,4-D, aminopyralid, and aminocyclopyrachlor were sprayed over top of non-auxin resistant soybeans at 0.001X, 0.01X, and 0.1X of their standard labeled rate to simulate tank contamination or possible particle drift. Visual evaluations, leaf samples, and high-quality images were taken at 3, 7, 14, and 21 days after application with hopes of discerning between herbicide symptomologies. Leaf samples were analyzed using the liquid chromatography-mass spectrometry to determine herbicide concentration. Herbicide concentration in leaf samples decreased while symptomology increased over time. All herbicides exhibited similar symptomology consisting of cupping of the leaves, epinasty in the petiole, and curling of the leaves. At 21 DAT, all 0.1X treatments had greater than 20% crop injury with 2,4-D indicating the least. Auxin herbicides can reduce yields at low concentrations. These herbicides have the same mode of action which makes discerning symptomology difficult even if

the exact dosage and time frame is known. Analytical analysis is needed to differentiate between auxin herbicide damages.

Nomenclature: aminocyclopyrachlor, aminopyralid, 2,4-D, dicamba, soybean (*Glycine Max* L.)

Keywords: drift, tank contamination, crop injury

Introduction

Auxin herbicides starting with 2,4-D are not new in today's agriculture, dating back to the 1940's (Peterson 1967). These Group 4 herbicides are synthetic chemicals that mimic naturally occurring auxins in dicot species. They are primarily used to control weeds in monocot systems but plant breeding and advancements in technology has allowed for transgenic resistance in certain crops to withstand a normally lethal dose of a specific auxin herbicide (Behrens et al. 2007; Waltz 2010; Wright et al. 2010). The introduction of dicamba-resistant soybeans (Bayer Crop Science, St Louis, MO, USA) and 2,4-D resistant soybeans (Corteva, Indianapolis, IN, USA) in 2016 and 2019, respectively, has directly corresponded to an increased use of dicamba and 2,4-D (Barber et al. 2015; Norsworthy et al. 2015).

Although these auxin-resistant soybean varieties have allowed growers to provide better weed control resulting in increased yields, there is a trade-off in greater potential for off-target movement (OTM). Susceptible soybean varieties can be injured from tank contamination or vapor/spray drift (Bovey and Meyer 1981; Solomon and Bradley 2014; Wax et al. 1969). 2,4-D and dicamba have volatile formulations that can result in OTM (Behrens and Lueschen 1979; Egan and Mortensen 2012; Grover et al. 1972). Other auxins also have the biological potential to injure soybeans if they are exposed to soybeans (Anonymous 2017; Anonymous 2019).

Commonly used auxin herbicides that are to be included in this project include aminocyclopyrachlor (ACP) as Method™ (Bayer Crop Science), 2,4-D as Weedar 64™ (Nufarm), dicamba as Clarity™ (BASF), and aminopyralid as Milestone™ (Corteva).

POST emergence herbicides in agronomic settings have shown an increase in product use since the introduction of herbicide tolerant crops. Common herbicide tolerant soybean crops include Xtendimax™ (dicamba and glyphosate tolerant), Enlist E3™ (2,4-D choline, glyphosate, and glufosinate tolerant), Roundup Ready™ (glyphosate tolerant), LibertyLink™ (glufosinate tolerant), and Credenz™ (glufosinate and glyphosate tolerant). POST herbicides allow for an application over top on already emerged crop with little crop response. With the introduction of glyphosate-tolerant soybeans, the amount of glyphosate active ingredient (ai) used in the United States increased from 18,144,000 kg ai to 125,384,000 kg ai in 1995 and 2014, respectively (Benbrook 2016).

Aminocyclopyrachlor, commonly referred to as ACP, is utilized on rights-of-way, pastures, industrial areas, and roadsides. ACP is used for the control of annual and perennial broadleaf and brush species in non-crop areas (Anonymous 2017). The mechanism of action for ACP is a plant growth-regulator while being highly mobile in the environment as well (Shaner 2014). Soybeans are highly sensitive to ACP through direct contact or runoff situations (Strachan et al. 2011). ACP has a vapor pressure of 4.89 $X10^{-6}$ kPa (Strachan et al. 2010).

Aminopyralid is a pasture and non-crop herbicide similar to ACP in the areas of which it can be sprayed (Anonymous 2019). Aminopyralid creates similar symptoms to ACP in soybeans (Strachen et al. 2013). The vapor pressure of aminopyralid is 2.56 $X10^{-11}$ kPa which is less compared to ACP (U.S. EPA 2020). In saturated soils, aminopyralid is weakly adsorbed which can lead to implications of runoff situations (Shaner 2014). This herbicide is considered to be relatively nonvolatile (Senseman 2007).

2,4-D amine can be used in multiple crop species as well as non-crop situations for the control broadleaf weeds. The label for Weedar 64™ indicates that it can only be used in soybeans as a pre-plant, and 2,4-D is not to be sprayed within 15-30 days of planting depending on application rate (Anonymous 2016). This chemical is mobile in soil, but rapid degradation decreases its likelihood of leaching (Shaner 2014). The vapor pressure of 2,4-D is 1.87 $X10^{-8}$ kPa at 25 C which is more similar to ACP than aminopyralid (U.S. EPA; 2005). Over 600 products containing 2,4-D are in commerce (Song 2014).

Dicamba can be used as a burndown, preplant, preemergence, and/or postemergence in dicamba-tolerant cotton and soybeans to control broadleaf weeds (Anonymous 1997). Dicamba-tolerant crops have aided in the battle of glyphosate resistant weeds in crops such as palmer amaranth (*Amaranthus palmeri*) as well as others (Meyer et al. 2015). Visual symptoms from dicamba applied at 0.01% of the labeled rate can occur on soybeans (Scholtes et al. 2019, Steckel et al. 2005). Dicamba has a vapor pressure of 4.5 $X10^{-6}$ kPa at 25 C which is the highest of the 4 herbicides to be used in this field study. Similarly to 2,4-D, dicamba is also mobile in soil, but rapidly degrades (Shaner 2014).

Tank contamination can contribute to injured soybeans even if only trace amounts are present (Andersen et al. 2004; Scholtes et al. 2019). Dicamba and 2,4-D have been proven to be volatile (Egan and Mortensen 2012; Behrens and Lueschen 1979; Mueller et al. 2013; Sosnoskie et al. 2015; Strachan et al. 2013). There have been instances of off-target movement indicating that dicamba and 2,4-D can cause injury to non-targeted crops, fruits, vegetables, and woody plant species due to spray or vapor drift (Culpepper et al. 2018; Dintelmann et al. 2020; Hemphill and Montgomery 1981; Mohseni-Moghadam and Doohan 2015).

Particle drift occurs when herbicide spray particles never reach their intended target and is affected by spraying in windy conditions and other factors. Vapor drift or volatilization is the result of an herbicide, after reaching the intended target, becoming volatile and moving off-target (Bedos et al. 2002; Strachan et al. 2013). Dicamba volatilization can be affected by temperature and pH (Mueller and Steckel 2019). Dicamba and 2,4-D are the only herbicides out of these 4 that have a labeled use as a preplant burndown. Following all guidelines on the label is imperative to reducing OTM and injury in already emerged soybean plants.

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 present at emergence to early pod soybean growth stages directly correlate with reduced yield and increased symptomology with the three to four leaf stage exhibiting most severe symptomology (Auch and Arnold 1978; Griffin et al. 2013; Jones et al. 2019; Marple et al. 2008; Wax et al. 1969).

The post-emergence application was designed to compare the effects of four auxin herbicides applied at three rates to simulate tank contamination on non-auxin tolerant soybeans. The 1st objective of this study was to visually differentiate symptomology caused by ACP, 2,4-D, dicamba, and aminopyralid from POST applications. To do this, high quality images were taken of 1 of the 2 trifoliate leaves collected from each treatment (TRT) at 1, 3, 7, 14, and 21 days after treatment (DAT). The 2nd objective was to harvest individual plots and determine the effect on yield. The 3rd objective was to quantify the specific herbicide present in the leaf and relate it to the amount of injury sustained.

Diagnosing soybean symptomology can be a challenge. The aim of this research is to better understand the symptomology caused by ACP, 2,4-D, dicamba, and aminopyralid when sprayed over the top of non-auxin resistant soybeans. These four auxin type herbicides are commonly used within the same vicinity of one another which can alternatively harm surrounding plants. When diagnosing herbicide symptomology in soybeans it is imperative to check the immediate location, identify herbicides used by the applicator, as well as know the surrounding area and which herbicides may be sprayed there. County agents and cooperators may gain from this research to better identify auxin herbicide symptomology in soybeans. Changes in herbicide formulations, environmental factors, and soybean genetics may cause the herbicides to cause different effects.

Materials and Methods

Field experiments:

Research was conducted in Knoxville, TN in 2019 and 2020 at the East Tennessee Agresearch and Education Center. The area to conduct this experiment utilized tilled ground as the medium to plant glufosinate tolerant soybeans (Pioneer P47A76L) at 76 cm row spacing using a pull behind type planter. Each plot consisted of 6 rows measuring 9.1 meters in length. Only 6 meters of the 2 center rows (labeled replicate A and B) of each plot received a TRT which allowed 1.5 meters on all sides of the treated area for a border.

There were 3 field studies, 2 with full sampling and photography, 1 with only crop response and yield (Table 1). This project utilized a randomized complete block design with three replications. Visual evaluation and yield were separated using means separation and LSD. Concentration data from lab analysis was regressed over time and correlated to observed visual injury.

The herbicides examined include aminocyclopyrachlor (ACP), 2,4-D, dicamba, and aminopyralid. The standard labeled rates (SLR) used to base the application rates are 0.12 kg ae ha^{-1} for ACP and aminopyralid while dicamba and 2,4-D are used at 1.12 kg ae ha^{-1}. These SLR's approximate the use rates under actual field conditions. Dicamba and 2,4-D are typically used in agronomic settings often as a POST emergent product applied to soybeans and cotton. ACP and aminopyralid are not labeled for agronomic use, but are used in pastures, rights-of-way, and brush control (Anonymous 2017; Anonymous 2019). Lower use rates of ACP and aminopyralid reflect intrinsically more active herbicides. A wide divergence of rates was used. Each of the POST herbicides were applied individually at the R1 soybean growth stage (first bloom) at three different rates: 0.1X, 0.01X, 0.001X of the SLR. To mix each herbicide for the application, a stock solution was made for each herbicide, and then a serial dilution technique was used, with 1 mL, 10 mL, and 100 mL of each mixture was placed into a 1000 mL bottle containing 999 mL, 990 mL, and 900 mL of water to correspond to the 3 different rates. All bottles contained 1000 mL of the respective herbicide mixture after mixing.

Prior to POST application, a 10 cm filter paper (Whatman #1) was placed atop the canopy for application verification. This area was not sampled for the remainder of the study. The filter paper for each plot was collected and placed into a labeled 50 mL conical tube, placed into a cooler, then transferred to a freezer at -18° Celsius for later analysis. The application was made using a CO_2 pressurized backpack sprayer with an operating pressure of 275 kPa and a 4-nozzle boom. The nozzle bodies were spaced 51 cm apart utilizing 8002 Flat Fan nozzles for optimum application coverage. All applications were sprayed at 187 L/ha. After each treatment was sprayed, there was a designated area away from all plots to clean the system using a self-made containment system made from 10 cm PVC pipe with a rubber slot for each nozzle to rinse into. Care was taken to not cross contaminate plots. TRT's were sprayed from low to high concentrations, and between each herbicide the boom was cleaned by spraying a 500 mL solution of 50% acetone/water followed by 1000 mL of water.

Plots were evaluated for herbicide response at 1, 3, 7, 14, and 21 days after treatment (DAT). A scale of 0-100 was used to assess crop injury with 0 being no injury and 100 being complete necrosis (Sciumbato et al. 2004). Plant response varied from leaf cupping and crinkling, galls, epinasty, leaf curling, and complete necrosis (Al-Khatib and Peterson 1999; Andersen et al. 2004; Auch and Arnold 1978; Wax et al. 1969). Border rows for each plot were inspected for injury at respective sampling intervals to assess potential drift between plots.

Sample Collection and Image Capture

Two trifoliate leaves (one from each of the two center rows labeled A and B) were collected after the herbicide response had been evaluated. The trifoliate leaf that came from row 'A' was placed directly into a Whirl-Pak™ sampling bag with a label corresponding to the study, plot, row (A), and date. The trifoliate leaf that comes from row 'B' was placed onto a sheet of paper in a modified tote for a high-quality photo to be obtained. A constant battery powered light source was placed inside the tote to allow for uniform lighting across all photos. The lid was modified to allow the high-resolution camera lens to fit in the lid, being careful to block outside sunlight from entering the tote. Samplings and photos were taken early morning for all sampling periods. After the photo was obtained, the trifoliate leaf was placed into its corresponding bag. All trifoliate leaf samples were placed in a cooler until being transferred to a freezer at -18° Celsius.

Gloves and the sheet of paper for photos were changed between each plot to avoid possible contamination.

Laboratory methods

Soybean leaf samples were homogenized using a mortar and pestle with liquid nitrogen poured onto the sample. Each trifoliate leaf (3 leaflets) was composited into that sample. 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 into a freezer at -18° Celsius. After all samples have been homogenized and placed in 50 mL conical tubes, then 30 mL of acetonitrile was added to tubes and shaken overnight (˜16 hr). The following morning ACP and aminopyralid samples were taken off the shaker and filtered. Dicamba and 2,4-D samples received 500 µL NaOH, sat at room temperature (21°C), received 500 µL HCL, heat in 60°C water bath for 30 minutes, allowed to cool, and centrifuged in a Thermo Scientific Rocket Evaporator™. 10 mL luer-lock tip syringes with a 25 mm syringe filter were used to filter extracts into 2 mL HPLC vials with corresponding labels. The 2 mL HPLC vials with the sample inside were placed in the LC/MS for analysis.

Lab analysis used an Agilent 1260 liquid chromatography coupled with a Agilent 6470 mass spectrometry. All separations used a gradient of water + 0.1% formic acid and acetonitrile + 0.1% formic acid (all reagents MSMS grade). Details of the analysis including stationary phase, parent ions, confirmatory ions, and other details were based upon other published sources or were derived in-house (Table 2). Soybean leaves from non-treated plots were analyzed to verify that no auxin herbicides were present, and also leaves were fortified with known amounts of each auxin herbicide and the recovery determined. Recoveries from these fortified samples ranged from 57 to 98% (Table 2). Concentration data presented have been corrected for recoveries.

Data analysis

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

The herbicide concentration data was fit to the equation:

EQ1 $f = a*\exp(-b*x)$

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 is a two parameter, single exponential decay model; fit most of the data curves relatively well with some notable exceptions.

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

EQ 2 $y = mx+b$

where m represents the slope of the line and b represents the y-intercept, or potentially initial injury observed. The regression analyses were performed using Sigmaplot V 14.0.

Results and Discussion

Soybean Response

Environmental conditions can affect plant responses to various herbicides, and auxins are no exception. Rainfall and soil conditions were conducive to optimum soybean growth as indicated by a yield of greater than 4000 kg ha^{-1} (Table 3). In general, all four herbicides caused similar soybean responses and had similar effects due to application dosage. As expected, as the herbicide dose increased there was much more soybean response, which was characterized as leaf malformation and necrosis in more extreme observations. The general ranking of the relative soybean injury caused by the auxin herbicides in this research at these rates was 2,4-D < ACP < aminopyralid < dicamba. Each herbicide will be discussed individually, and for discussion purposes the full reference dose or 1X rate will be stated at the start of each herbicides discussion. Readers are reminded that the actual applications were stepwise order of magnitude decreases in the actual applications. The addition of non-ionic surfactant at 0.25% v/v also probably increased herbicidal effects.

The full reference dose for ACP was 105 g ha^{-1}. Soybean injury from ACP increased over time and at the relative dose was less than other auxin herbicides (Table 3). The lowest ACP dose did not reduce soybean yield compared to the nontreated control.

The full reference dose for aminopyralid was 102 g ha^{-1}. Soybean injury increased over time at all dose levels (Table 3). The lowest aminopyralid dose did not reduce soybean yield compared to the nontreated control.

The full reference dose for 2,4-D was 1.06 kg ha^{-1}. The overall soybean response was much less from 2,4-D than any of the other treatments (Table 3). Soybean injury did not occur or was declining over time. Several of the treatments at the low dose showed no soybean response until 14 days after treatment, and injury after that was only negligible. Soybean yield was not reduced by either the low or medium rates, and only decreased by 12% at the highest dose. These findings are consistent with other researchers which reported 2,4-D was less injurious to soybeans compared to other auxins (Egan et al. 2014).

The reference dose for dicamba was 1.12 kg ha^{-1}. This is higher than the normal post-emergent use rate in soybeans, but this amount has been used in various agronomic situations. Also, the fact that we are diluting it via factors of 10, 100 and 1000 would indicate a biologically relevant range of doses. Another factor that might have affected the results was our use of the diglycol amine formulation with no drift control agents added. Dicamba at the lowest reference dose decreased soybean yield 28% (Table 3). Soybean response increased over time from 3 to 21 days after treatment for all doses, indicating the challenge of explaining soybean response as confounded by the time of application. Similar injury from a given herbicide would be observed from the same herbicide at a different time duration from that herbicide exposure. This idea will be discussed more in the next section on images collected.

Image comparisons

One of the major goals of this entire project was to collect a set of visual images of the various auxin herbicides that could be used to differentiate the different herbicides based solely on visual symptomology. After analysis of the images, this outcome may not be possible (Figure 1). As it turns out, symptomology of the different auxin herbicides slightly changes over time, so that a given herbicide affect soon after application from one herbicide will look quite similar to the effect a different herbicide has at a different time interval post exposure. It is often the situation where the exact time of herbicide exposure to a nontarget plant is unknown, thus complicating this objective.

All soybean responses from the four herbicides were similar in that higher application doses caused much more soybean response. We will attempt to characterize and describe the various herbicide responses and also provide representative images.

For ACP treatments in 2019, the low and medium rates exhibited mostly downward cupping of the leaves, stunting, and dark green foliage while the high rate resulted in epinasty of the petiole, dark green foliage, and necrosis of the terminal bud (Figure 2, 3). In 2020, the low rate showed mostly downward cupping, slight epinasty, and bumps on the younger leaves. The medium rate showed mostly downward cupping, more upward cupping than the low rate, slight epinasty, and bumps on the younger leaves. The high rate exhibited moderate epinasty, little upward cupping with no downward cupping, curling of the leaves and yellowing of the leaves (Figure 4).

For aminopyralid in 2019 the lowest rate exhibited exclusively downward cupping of the leaves (Figure 5). The medium rate showed mostly upward cupping with some downward (Figure 6). The highest rate showed epinasty in the petiole and curling of the leaf (Figure 7). In 2020 the lowest rate exhibited upward and downward cupping of the leaves and slight epinasty. The medium rate showed mostly upward cupping with some downward, moderate epinasty, and bumps on the leaves. The highest rate showed severe epinasty, bumps on leaves, little upward cupping, and curling of the leaf.

Images from the low rate of 2,4-D in 2019 showed little visual symptomology throughout the duration of the study which is consistent with previous research (Andersen et al. 2004). Some rates exhibited slight symptomology consisting of bumps on the leaves with the younger leaves showing more prominent bumps (Figure 8). The high rate showed severe epinasty and necrotic shoot and leaves. This is consistent with the symptomology observed in previous studies (Auch and Arnold 1978; Behrens and Lueschen 1979; Wax et al. 1969). Less leaf cupping was observed on the high rate when compared to the low and medium rates. The high rate showed epinasty in the petioles, some cupping, and leaf curling.

Dicamba response in the low and medium rates showed mostly upward cupping, slight stunting, and epinasty in the petioles (Figure 9, 10). The high rate showed severe epinasty and necrotic shoot and leaves (Figure 11). This is consistent with the symptomology observed in previous studies (Auch and Arnold 1978; Behrens and Lueschen 1979; Wax et al. 1969). Less leaf cupping was observed on the high rate when compared to the low and medium rates.

Herbicide Concentrations

ACP concentrations in the soybean leaf samples varied, but ultimately decreased over time (Figure 12) in 2 of 3 treatments, with no appreciable change in low dose plots. Samples taken at 7 DAT in 2019 showed greater variability than other sampling intervals, and the cause of this variability is unknown to the authors, but sampling error or storage anomalies would be possible causes. Soybean injury in these same plots in 2019 increased over time at the low and medium rates while staying consistent over time at the high rate (Figure 12). ACP concentration in the soybean leaf samples decreased over time in 2020 (Figure 13). Soybean injury increased over time for all rates.

Aminopyralid concentrations decreased over time in 2019 (Figure 14). Soybean injury increased over time for all aminopyralid rates. Aminopyralid showed similar responses in 2020

(Figure 15). Herbicide concentrations were consistent over time at the low rate while showing a decrease over time at the medium and high rates. Percent injury increased over time for all aminopyralid rates.

The dissipation curves of 2,4-D and the soybean response each year was similar. 2,4-D concentration decreased over time, usually rapidly after application (Figures 16, 17). Soybean injury was minimal (<5%) at the low and medium application rates, and peaked at ~35% 3 DAT and then slowly diminished over time (Figures 16, 17). The low rate showed little visual symptomology throughout the duration of the study which is consistent with previous research (Andersen et al. 2004).

Dicamba concentrations decreased in soybean leaf samples over time in 2019 and 2020 (Figures 18, 19). Herbicide concentration in the soybean leaves showed variation in dissipation rate in the low and medium rates while the high rate shows the herbicide dissipating consistently over time. Percent injury increased over time for all dicamba rates.

Filter papers placed at the top of the soybean canopy at the time of herbicide application provided the opportunity to quantify the actual herbicide applied to each plot (Table 4). Similar methods were used to the quantification of the herbicide analytes from the soybean leaves, but extraction from the filter papers was assumed to be complete (preliminary data not shown). The herbicide concentrations show the target dosage and the mean concentration normalized to the target dosage as a percent (Table 4). The standard error of the mean based on six measurements was also calculated. Recoveries for ACP, aminopyralid, 2,4-D, and dicamba were 97, 150, 79 and 118%, respectively (Table 4). The overall average recovery was 111% with a standard error of 20%. Given that the herbicides were applied over two orders of magnitude differences these measurements indicate good accuracy and precision in the field application.

Regression analysis of herbicide dissipation curves under field conditions is always a challenge, and these data were no different (Table 5). Several of the dissipation curves had a flat, nonresponsive change in herbicide concentration over time, and thus the r^2 values are low; these are often the lower concentrations of auxin such as 2019 ACP lowest rate, 2019 aminopyralid at middle rate, and dicamba 2019 at lowest rate (Table 5). The analysis was also complicated by the challenge that several of the 2019 dissipation curves had no sampling at the one DAT interval for the lowest herbicide rate. This was done in 2019 because only plots that had visual symptomology were sampled, and this change was made in 2020 so that all plots were sampled on 1 DAT. Many of the regression curves were fit nicely by the first-order equation with many r^2 values above 0.75 (Table 5). The Y intercept value is a surrogate measurement of the initial herbicide concentration in each treatment, and thus varied by orders of magnitude. The K and subsequent DT 50 values indicate the relative dissipation or loss of each herbicide in the soybean leaf. Most apparent dissipation curves were more rapid at the higher herbicide doses, which could be due to more translocation out of the treated leave from a larger concentration, more apparent degradation of each herbicide, or other factors given the complex interactions of auxin herbicides in sensitive soybean plants. Most of the herbicides showed more rapid dissipation at the higher doses applied (Table 5). All herbicides showed decreases in auxin herbicide concentrations over time with some having rapid dissipation with half-lives less than three days.

In contrast to the herbicide concentrations, the soybean responses showed a positive slope in most lines, with a few general exceptions of being flat or no slope (Table 5). Most ACP, aminopyralid, and dicamba treatments exhibited more soybean injury over time; and 2,4-D showed either no change or a slight decrease in herbicide response over time. The Y intercepts would serve as an indicator of initial plant response and also followed a stepwise order of magnitude differences in conjunction with the herbicide doses (Table 5).

The observation that the herbicide concentration in the treated leaves is rapidly declining while the apparent soybean responses increasing over time shows the dilemma of trying to obtain soybean leaf samples and verify which herbicide, if any, would be responsible for a given soybean response. It would be implausible given this observation to quantifiably and certainly identify which possible auxin herbicide would be causing the soybean response solely on visual symptoms. This is an example where herbicide chemical analysis would be more definitive in conclusively showing which herbicide caused the damage.

There were many constraints on this research and possible areas of improvement. For example, no metabolites were measured in any of the soybean leaves which would have been a good measurement to have, especially with respect to dicamba. The differential recoveries of the four herbicides also brings into question the precision of the analytical methods used. However, given that our recoveries from the filter papers were good over multiple orders of magnitude, and they were in good agreement with our soybean leaf samples, we have confidence in the extraction and analysis of the samples in this project. Given that our lowest application dosage still always had a major response, future research would be plausible to examine even lower rates of all the herbicides with the possible exception of 2,4-D. This research was conducted on a single soybean variety, and it is quite expected that different varieties and maturity groups may respond differently. The growing conditions in this study were conducive to rapid soybean growth, and different environmental conditions could possibly affect the results. Another aspect of this research is that it did not expose the soybeans in the same physical mechanism as a potential vapor drift could be if a chemical such as dicamba, or any of the other auxin herbicides, could be coming via landscape scale drift mechanisms. All of these matters imply that future research is still warranted.

Chapter 2

Pre-emergence Auxin Applications on Non-auxin Resistant Soybeans

Running title: Soybean response from PRE Auxins

Pre-emergence Auxin Applications on Non-auxin Resistant Soybeans

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Abstract

Some auxin herbicides are known to have residual activity within the soil. This can be beneficial in non-crop and some cropping systems if utilized correctly. Aminocyclopyrachlor (ACP), aminopyralid, 2,4-D and dicamba were sprayed as a pre-emergence application on non-auxin resistant soybeans at 53, 60, 530 and 560 g ha^{-1}, (1/2X of their standard labeled rate) to simulate a runoff situation or other event where the chemical leaves the treated area and moves to an adjacent area that may contain sensitive soybeans in water, manure, or sediment. Stand counts at 30 days after planting (DAP) and visual evaluations were made at 56, 60, 67, and 74 DAP. ACP and aminopyralid had the greatest effect on germination resulting in no germination and no yield for both herbicides. Dicamba reduced germination 85% while yielding 60% less than the non-treated. 2,4-D did not affect soybean germination, growth, or yield and was not different from the nontreated.

Nomenclature: aminocyclopyrachlor, aminopyralid, 2,4-D, dicamba, soybean (*Glycine Max* L.)

Keywords: tank contamination, crop injury

Introduction

Soil applied herbicides can have major effects on sensitive crops. Soils may be contaminated with auxin herbicides in several pathways, including water movement and over land flow from a treated area, manure that may contain herbicide residues, accidental direct application or herbicide drift or tank contamination.

Aminocyclopyrachlor (ACP) and aminopyralid are labeled for non-crop use, while dicamba and 2,4-D can be used in respective herbicide tolerant crops. Sensitive crops such as soybeans are susceptible to injury if ACP or aminopyralid are present in trace amounts in the soil (Shaner 2014, Strachan et al. 2011). 2,4-D and dicamba are also mobile in the soil but degrade reducing the likelihood of movement in the soil (Shaner 2014). The objective of this research was to compare relative soil activity to emerging soybeans for these 4 auxin herbicides.

Materials and Methods

Field experiments:

Research was conducted in Knoxville, TN in 2019 and 2020 at the East Tennessee Agresearch and Education Center. The area to conduct this experiment utilized tilled ground as the medium to plant glufosinate tolerant soybeans (Pioneer P47A76L) at 76 cm row spacing using a mechanical planter. Each plot consisted of 6 rows measuring 9.1 meters in length. Only 6 meters of the 2 center rows of each plot received the herbicide which allowed 1.5 meters on all sides of the treated area for a border.

There were 2 field studies to analyze the effects of ACP, 2,4-D, dicamba, and aminopyralid applied as a PRE application to non-tolerant soybeans (Table 6). A randomized complete block design was utilized with three replications. The rates for the soil applied application were 1/2X of the standard labeled rate (SLR). ACP and aminopyralid SLR considered was 0.12 kg ae ha^{-1}, while 2,4-D and dicamba was 1.12 kg ae ha^{-1}. 1/2X rates were chosen to simulate soil movement runoff where the chemical leaves the treated area and moves in contaminated water, manure or sediment to an adjacent area that may contain sensitive soybeans.

Prior to the PRE application, a 10 cm filter paper (Whatman #1) was placed directly on the ground in each of the plots to be treated. The filter paper was collected and placed into a corresponding labeled 50mL conical tube, placed into a cooler, then transferred to a freezer at -18º Celsius. These filter papers were analyzed for herbicide application verification.

The application was made using a CO_2 pressurized backpack sprayer with an operating pressure of 275 kPa and a 4-nozzle boom. The nozzle bodies were spaced 51 cm apart utilizing 8002 Flat Fan nozzles for optimum application coverage. All applications were sprayed at 187 L/ha. After each of the treatments were sprayed, the spray equipment was cleaned in an area spatially away from the plots using a self-made containment system made from 10 cm PVC pipe with a rubber slot for each nozzle to rinse into. The boom was rinsed by spraying 500 mL solution of 50% acetone/water followed by 1000 mL of water. The PRE applications were made 1 day after planting, and field then irrigated with 1.27 cm of water. Each of these plots were evaluated to indicate percent emergence and damage at 56, 60, 67, and 74 DAP. A scale of 0-100 was used to assess inhibited emergence with 0 being no hinderance and 99 being no emergence. Soybean stand counts were conducted for each plot approximately 3 weeks after soybean emergence (Table 7). Soybeans were counted in 2 rows, each 6 meters in length.

Data analysis

Statistical analysis was conducted using Agriculture Research Management (ARM) Software. Mean separation for stand counts, visual evaluations, and yield differences was performed using Fisher's protected LSD at $P \leq 0.05$.

Results and Discussion

Soybean Response

Auxin herbicide effects varied when applied as a PRE in relation to soybean response. ACP and aminopyralid resulted in zero germination in all plots (Table 7, Figure 20, 21). There was some movement of ACP, aminopyralid, and dicamba in the soil as boarder rows were substantially impacted by herbicide movement (Figure 20, 21, 22). ACP and aminopyralid had the greatest soil effect of these auxins and need careful consideration when being used around crops or where crops are intended to be planted.

Dicamba applied as a PRE resulted in substantial germination reduction but did allow some soybean emergence (Table 7, Figure 22). The plants that emerged in the area treated with dicamba did not show significant symptomology later (Data not shown). 2,4-D had little to no effect on soybean emergence as yields were statistically the same as the UTC (Figure 23, Table 7). Little or no damage from 2,4-D applied as a PRE was consistent with previous research findings and it is also noted that if visual injury was observed, the plants would grow out of it (Soltani et al. 2008).

Three of the four herbicides had similar effects when used as a PRE with the exception being 2,4-D. ACP and aminopyralid treated plots resulted in 100% yield loss, dicamba 60% reduction in yield, and 2,4-D was statistically the same as the UTC (Table 7).

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Appendix

Table 1. Field sites with planting, application, and harvest dates. All trials were conducted in Knoxville, Tennessee.

Year	Planting date	Application date	Harvest date
		Post	
2019	6/3/2019	7/12/2019	10/9/2019
2020	5/14/2020	7/6/2020	10/6/2020
2020[1]	6/1/2020	7/21/2020	11/11/2020

[1] no leaf samples or digital images were captured from this trial.

Table 2. Parameters for LC/MS analytical methods.

Compound	**Analytical Column**	**Parent Ions**	**Confirmation Ions**	**Retention time**	**Limit of Detection**	**Recovery**
		(m/z)	(m/z)	min	ppb	%
Aminocyclopyrachlor	Amino	214	196 168 101 68.1	1.8	0.25	57
Aminopyralid	Phenyl-Hexyl	207 206.8	188.9 160.9 133.9 161	1.27	1	75
2,4-D	Phenyl-Hexyl	219	160.9 125	4.6	0.5	98
Dicamba	Phenyl-Hexyl	221 219	177 175 145	3.9	0.5	75

Table 3. Effect of auxin herbicides applied POST to soybeans from 3 to 21 days after treatment, and soybean yield at end of season from 3 trials conducted in Knoxville, TN. [a,b]

Herbicide	Dose[c]	3 DAT		7 DAT		14 DAT		21 DAT		Yield	
	%	-------------- soybean injury, % ---------------								$kg\ ha^{-1}$	
ACP	0.001	2.1	FE	8.2	G	12.8	F	11.7	H	4136	AB
ACP	0.01	7.7	DC	14.3	F	26.1	E	28.9	F	3254	C
ACP	0.1	38.2	C	44.3	C	50	C	57.8	C	583	E
Aminopyralid	0.001	1.6	FE	8.2	G	11.7	GF	13.9	H	3955	AB
Aminopyralid	0.01	11.6	C	17	FE	28.3	E	37.2	E	2520	D
Aminopyralid	0.1	49.9	A	57.6	B	70.6	B	73.3	B	182	EF
2,4-D	0.001	0	F	0	H	1.4	H	2.3	I	4234	A
2,4-D	0.01	4.4	DE	5.9	G	7.2	GF	3.9	I	4224	A
2,4-D	0.1	33.8	B	30.4	D	28.3	E	20	G	3778	B
dicamba	0.001	4	DE	17	FE	26.7	E	30	F	3116	C
dicamba	0.01	12.1	C	20.4	E	35	D	46.1	D	2319	D
dicamba	0.1	52.1	A	73.2	A	92.8	A	96.1	A	49	F
none, UTC										4311	A
LSD (0.05)		4.2		5		4.98		5.4		403	

[a] Means not followed by a common letter are significantly different ($P < 0.05$).

[b] Abbreviations: DAT, days after treatment; ACP, aminocyclopyrachlor

[c] Dose is expressed as a comparison to the X or full dose (see text for details).

Table 4. Herbicide concentrations based upon field samples collected at the time of herbicide application. Values represent the mean of 3 replications ± calculated standard error for those 3 observations. [a]

Year	Herbicide	Rate		Percent of applied		Average for each herbicide	
		%	g ha^{-1}	mean	Std Error		
2019	ACP	0.001	0.11	97.9	15.7		
2019	ACP	0.01	1.05	88.2	11.6		
2019	ACP	0.1	10.5	103.6	19.7		
2020	ACP	0.001	0.11	97.6	27.7		
2020	ACP	0.01	1.05	97.1	12.0		
2020	ACP	0.1	10.5	95.7	20.5	96.7	17.9
2019	Aminopyralid	0.001	0.12	136.0	20.9		
2019	Aminopyralid	0.01	1.23	128.1	41.7		
2019	Aminopyralid	0.1	10.2	192.6	43.7		
2020	Aminopyralid	0.001	0.12	134.1	14.1		
2020	Aminopyralid	0.01	1.23	147.8	19.9		
2020	Aminopyralid	0.1	10.2	160.5	3.5	149.9	24.0
2019	2,4-D	0.001	1.06	75.9	4.2		
2019	2,4-D	0.01	10.6	94.9	3.7		
2019	2,4-D	0.1	106	65.5	15.3		
2020	2,4-D	0.001	1.06	79.0	13.3		
2020	2,4-D	0.01	10.6	79.1	10.5		
2020	2,4-D	0.1	106	79.6	16.5	79.0	10.6
2019	dicamba	0.001	1.12	125.8	21.3		
2019	dicamba	0.01	11.2	114.4	22.0		
2019	dicamba	0.1	112	154.1	29.9		
2020	dicamba	0.001	1.12	129.9	47.7		
2020	dicamba	0.01	11.1	101.4	14.8		
2020	dicamba	0.1	112	84.6	39.7	118.4	29.2
all	all	all	all	111.0	20.4		

[a] Abbreviations: ACP, aminocyclopyrachlor

[b] Dose is expressed as a comparison to the X or full dose (see text for details).

Table 5. Herbicide descriptors and regression parameter values for change in herbicide concentrations and soybean response from 0 to 21 days after treatment. Data values are presented in Figures 12 to 19. [a]

			Herbicide concentrations				Soybean injury 3 to 21 days after treatment		
Year	Herbicide	Dose[b]	r^2	Y int	K	DT50	r^2	Y int	Slope
		%		ng g^{-1}		day		%	
2019	ACP	0.001	0.002	2	0.001	NS	0.51	1.9	0.5
2019	ACP	0.01	0.13	31	0.0477	14.5	0.81	8.5	0.72
2019	ACP	0.1	0.74	992	0.2818	2.5	0.17	51	-0.22
2020	ACP	0.001	0.9	27	0.9766	0.7	0.49	0.9	0.69
2020	ACP	0.01	0.82	197	0.7114	1.0	0.46	2.2	1.17
2020	ACP	0.1	0.99	22188	0.5035	1.4	0.76	30	1.63
2019	aminopyralid	0.001	0.44	12	0.1057	6.6	0.63	0.6	0.46
2019	aminopyralid	0.01	0.09	29	0.0488	14.2	0.68	13	0.79
2019	aminopyralid	0.1	0.99	304	0.5998	1.2	0.82	58	1.08
2020	aminopyralid	0.001	0.001	4.3	0.001	NS	0.76	0	0.66
2020	aminopyralid	0.01	0.11	26	0.0468	14.8	0.72	4	1.27
2020	aminopyralid	0.1	0.99	852	0.2596	2.7	0.67	44	1.51
2019	2,4-D	0.001	0.94	2.3	0.0987	7.0	0.12	0	0.03
2019	2,4-D	0.01	0.99	1190	1.6425	0.4	0.54	12	-0.34
2019	2,4-D	0.1	0.99	19199	1.6474	0.4	0.87	42	-1.03
2020	2,4-D	0.001	0.99	73	0.9296	0.7	0.01	1	0.01
2020	2,4-D	0.01	0.98	663	0.9916	0.7	0.01	2.7	0.09
2020	2,4-D	0.1	0.99	7618	0.652	1.1	0.38	36	-0.59
2019	dicamba	0.001	0.1	15	0.034	20.4	0.82	0	1.24
2019	dicamba	0.01	0.55	410	0.0955	7.3	0.95	11	1.36
2019	dicamba	0.1	0.97	8536	0.6289	1.1	0.64	59	2.05
2020	dicamba	0.001	0.32	24	0.0835	8.3	0.62	3	1.66
2020	dicamba	0.01	0.97	345	0.3628	1.9	0.84	2	2.26
2020	dicamba	0.1	0.69	13687	0.5308	1.3	0.88	46	2.66

[a] Abbreviations: ACP, aminocyclopyrachlor

Table 6. Field sites with planting, preemergence application, and harvest dates. All trials were conducted in Knoxville, Tennessee.

Year	Planting date	Application date	Harvest date
		PRE	
2019	6/3/2019	6/4/2019	10/9/2019
2020	5/14/2020	5/15/2020	10/6/2020

Table 7. Stand counts (plants within 1 meter), effect of auxin herbicides applied PRE to soybeans from 56 to 74 days after planting (DAP), and soybean yield at end of season from 2 trials conducted in Knoxville, TN. [a,b]

Herbicide	Dose[c]	Stand count		56 DAP		60 DAP		67 DAP		74 DAP		Yield	
				-------------soybean injury, %-------------								kg ha^{-1}	
ACP	1/2x	0	C	99	A	99	A	99	A	99	A	0	C
Aminopyralid	1/2x	0	C	99	A	99	A	99	A	99	A	0	C
2,4-D	1/2x	18	A	2.5	C	1.7	C	2.5	C	2.5	C	3874	A
Dicamba	1/2x	3	B	74.2	B	74.2	B	76.7	B	69.2	B	1614	B
none, UTC		20	A	0	C	0	C	0	C	0	C	4129	A

[a] Means not followed by a common letter are significantly different (P < 0.05).

[b] Abbreviations: DAP, days after planting; ACP, aminocyclopyrachlor

[c] Dose is expressed as a comparison to the X or full dose (see text for details).

Table 8. Herbicide treatment list with corresponding dose and application code. Application code A is preemergence and application code B is R1 stage. [a]

Trt #	Treatment name	Rate		Application code
1	Non-treated			
2	Aminocyclopyrachlor	0.123	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
3	Aminocyclopyrachlor	1.23	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
4	Aminocyclopyrachlor	12.3	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
5	2,4-D	1.12	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
6	2,4-D	11.2	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
7	2,4-D	112	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
8	Dicamba	1.12	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
9	Dicamba	11.2	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
10	Dicamba	112	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
11	Aminopyralid	0.123	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
12	Aminopyralid	1.23	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
13	Aminopyralid	12.3	g ae/ha^{-1}	B
	NIS	0.025	% v/v	B
14	Aminocyclopyrachlor	57	g ae/ha^{-1}	A
15	2,4-D	546.5	g ae/ha^{-1}	A
16	Dicamba	546.5	g ae/ha^{-1}	A
17	Aminopyralid	57	g ae/ha^{-1}	A

[a] Abbreviations: NIS, non-ionic surfactant

Table 9. 2019 Post emergence visual injury ratings and yield data. [a]

Herbicide	Dose[b]	Visual Injury (0-100%)				Yield
		3 DAT	7 DAT	14 DAT	21 DAT	kg ha^{-1}
ACP	0.001X	0	5	10	10	4277
		0	10	15	10	4701
		0	10	10	10	4371
ACP	0.01X	15	15	20	20	3470
		10	10	20	25	4243
		10	10	20	25	4069
ACP	0.1X	50	50	40	40	881
		50	50	50	50	968
		50	50	50	50	962
Aminopyralid	0.001X	0	5	5	10	3867
		0	5	5	10	3995
		0	10	10	10	4398
Aminopyralid	0.01X	15	15	20	25	2784
		20	20	30	35	2495
		15	15	20	30	2771
Aminopyralid	0.1X	60	70	80	80	262
		60	60	75	80	134
		60	65	75	75	303
2,4-D	0.001X	0	0	0	0	4714
		0	0	1	0	4492
		0	0	2	1	5259
2,4-D	0.01X	15	10	5	5	4183
		10	5	5	5	4465
		10	10	10	5	4660
2,4-D	0.1X	40	40	30	20	4304
		40	30	30	20	3793
		40	30	30	20	4761
Dicamba	0.001X	0	5	20	30	3685
		0	15	20	20	3988
		2	10	20	20	3705
Dicamba	0.01X	25	20	30	40	2616
		15	15	30	40	3194
		15	20	30	40	2677
Dicamba	0.1X	50	90	95	95	141
		60	90	95	95	148
		50	75	95	95	155
none, UTC		0	0	0	0	5044

Table 9 continued

	0	0	0	0	4412
	0	0	0	0	4607

[a] Abbreviations: DAT, days after treatment; ACP, aminocyclopyrachlor

[b] Dose is expressed as a comparison to the X or full dose (see text for details).

Table 10. 2020 Post emergence visual injury ratings and yield data. [a]

Herbicide	Dose[b]	Visual Injury (0-100%)					Yield
		1 DAT	3 DAT	7 DAT	14 DAT	21 DAT	kg ha^{-1}
ACP	0.001X	0	0	5	10	15	3968
		0	0	5	20	10	3477
		0	0	10	20	10	3363
ACP	0.01X	5	0	10	20	25	2233
		0	0	15	40	20	2933
		5	5	5	30	15	1469
ACP	0.1X	15	30	45	50	65	0
		15	30	40	65	50	281
		15	35	40	60	65	0
Aminopyralid	0.001X	0	0	5	10	10	4522
		0	0	5	10	15	2385
		0	0	5	15	10	3566
Aminopyralid	0.01X	5	5	15	25	35	2776
		5	5	10	30	25	2218
		5	5	15	30	20	2972
Aminopyralid	0.1X	25	40	50	80	75	0
		30	40	60	70	65	0
		35	50	60	70	70	234
2,4-D	0.001X	0	0	0	0	0	4184
		0	0	0	0	0	3907
		0	0	5	5	0	3687
2,4-D	0.01X	15	0	5	5	0	4521
		10	0	5	20	0	4408
		20	0	0	10	0	4519
2,4-D	0.1X	25	35	30	30	25	3660
		25	30	30	40	20	3496
		30	35	30	35	15	3459

Table 10 continued

Dicamba	0.001X	0	0	15	30	40	2256
		0	0	25	45	35	2767
		0	5	15	30	20	1846
Dicamba	0.01X	15	10	25	40	55	1329
		5	5	10	40	40	1459
		10	5	15	45	40	1426
Dicamba	0.1X	35	50	65	95	95	0
		30	50	60	95	95	0
		25	50	65	90	95	0
none, UTC		0	0	0	0	0	4403
		0	0	0	0	0	3869
		0	0	0	0	0	2457

[a] Abbreviations: DAT, days after treatment; ACP, aminocyclopyrachlor

[b] Dose is expressed as a comparison to the X or full dose (see text for details).

Table 11. 2020 Post emergence visual injury ratings and yield data from second location. [a]

Herbicide	Dose[b]	Visual Injury (0-100%)					Yield
		1 DAT	3 DAT	7 DAT	14 DAT	21 DAT	kg ha^{-1}
ACP	0.001X	0	5	10	10	10	4166
		0	10	10	15	10	4138
		0	15	15	10	10	4764
ACP	0.01X	0	10	15	30	40	2974
		5	15	30	30	40	4042
		5	15	25	30	40	3855
ACP	0.1X	15	30	40	50	70	681
		15	40	40	40	60	1036
		15	40	50	50	60	433
Aminopyralid	0.001X	0	10	15	20	15	3974
		5	5	15	15	15	4494
		0	10	15	20	20	4397
Aminopyralid	0.01X	5	15	15	40	60	1479
		10	20	30	35	50	2586
		5	15	25	30	45	2602
Aminopyralid	0.1X	15	50	50	70	70	0
		20	50	50	60	75	705
		15	50	60	60	60	0

Table 11 continued

2,4-D	0.001X	0	0	0	0	0	3288
		0	5	0	10	10	3869
		5	0	0	0	0	4705
2,4-D	0.01X	10	5	0	0	0	3433
		10	5	5	0	0	3905
		15	5	20	15	10	3923
2,4-D	0.1X	15	25	30	15	10	3277
		20	40	30	25	20	3423
		15	30	30	25	20	3829
Dicamba	0.001X	5	10	20	30	35	3139
		5	15	30	30	30	3376
		5	15	25	20	30	3278
Dicamba	0.01X	5	5	25	35	50	2612
		10	20	30	35	50	2566
		10	25	30	35	50	2995
Dicamba	0.1X	20	50	75	95	95	0
		20	60	70	90	95	0
		20	60	75	90	95	0
none, UTC		0	0	0	0	0	3796
		0	0	0	0	0	5129
		0	0	0	0	0	5081

[a] Abbreviations: DAT, days after treatment; ACP, aminocyclopyrachlor

[b] Dose is expressed as a comparison to the X or full dose (see text for details).

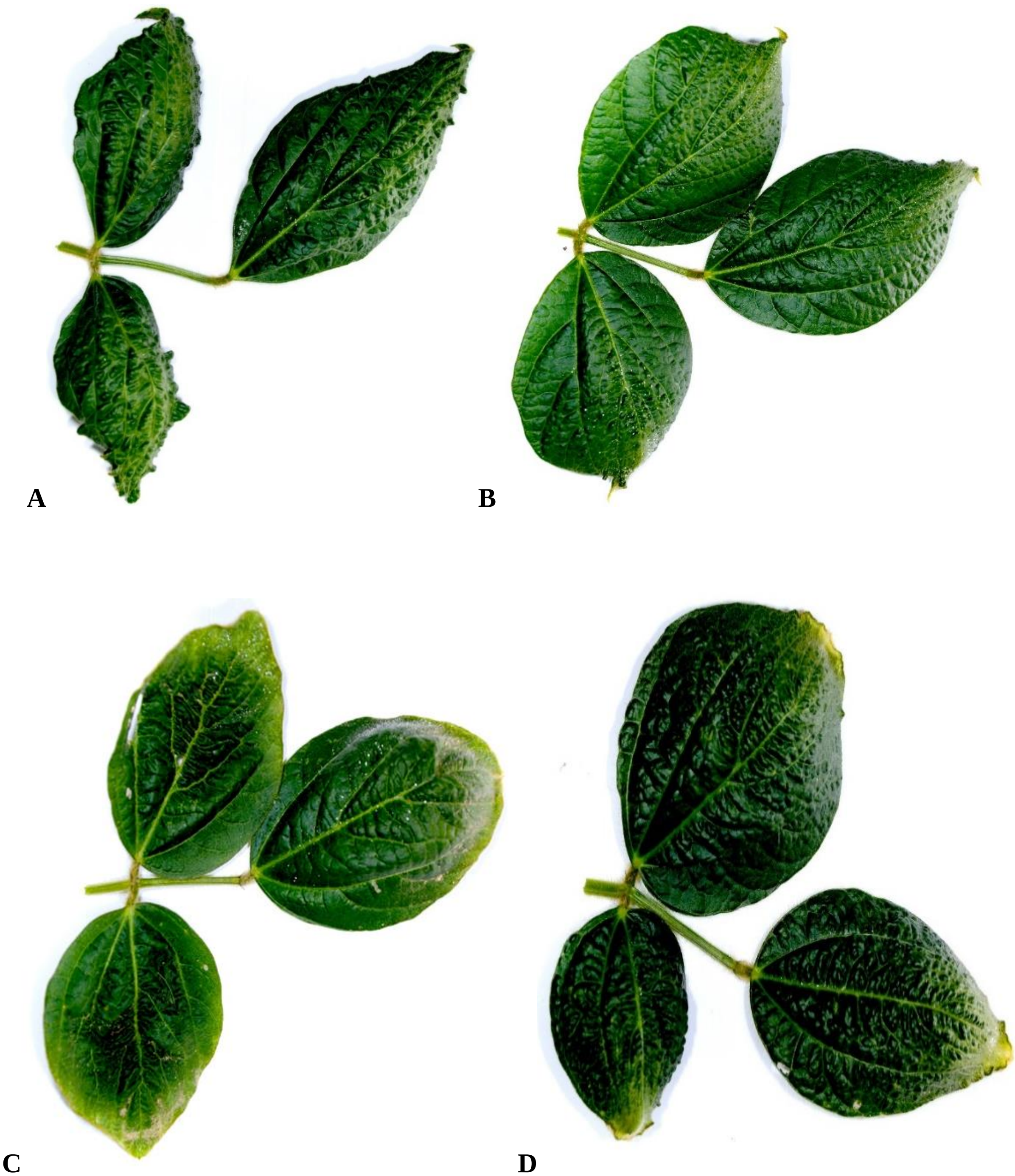

Figure 1. Visual similarities between herbicide symptomology and/or application rate. Image A, B, C, and D were taken 21 days after treatment. Image A is aminopyralid at 0.001X rate. Image B and D are aminocyclopyrachlor at 0.001X and 0.01X, respectively. Image C is dicamba at 0.001X rate.

Figure 2. Trifoliate image 21 days after treatment aminocyclopyrachlor at 0.001X rate.

Figure 3. Trifoliate image 21 days after treatment aminocyclopyrachlor at 0.01X rate.

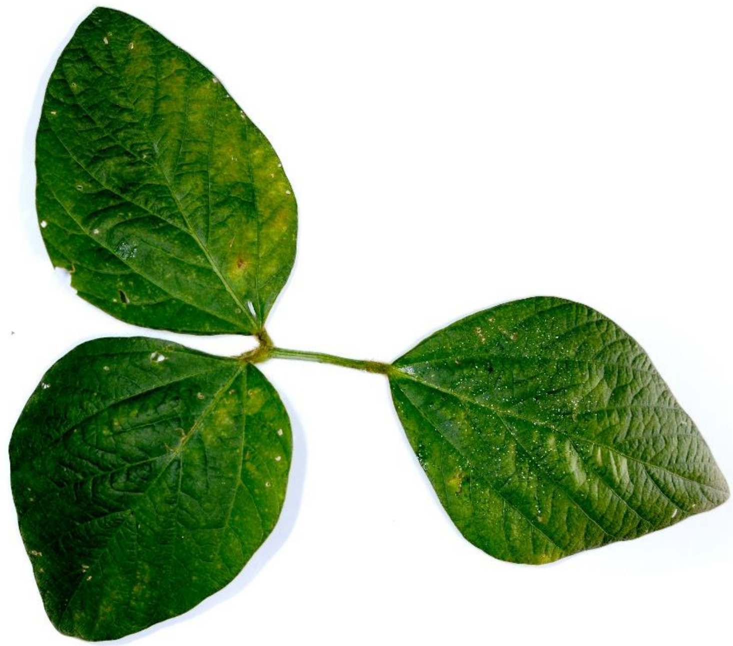

Figure 4. Trifoliate image 21 days after treatment aminocyclopyrachlor at 0.1X rate.

Figure 5. Trifoliate image 21 days after treatment aminopyralid at 0.001X rate.

Figure 6. Trifoliate image 21 days after treatment aminopyralid at 0.01X rate.

Figure 7. Trifoliate image 21 days after treatment aminopyralid at 0.1X rate.

Figure 8. Trifoliate image 21 days after treatment 2,4-D at 0.1X rate.

Figure 9. Trifoliate image 21 days after treatment dicamba at 0.001X rate.

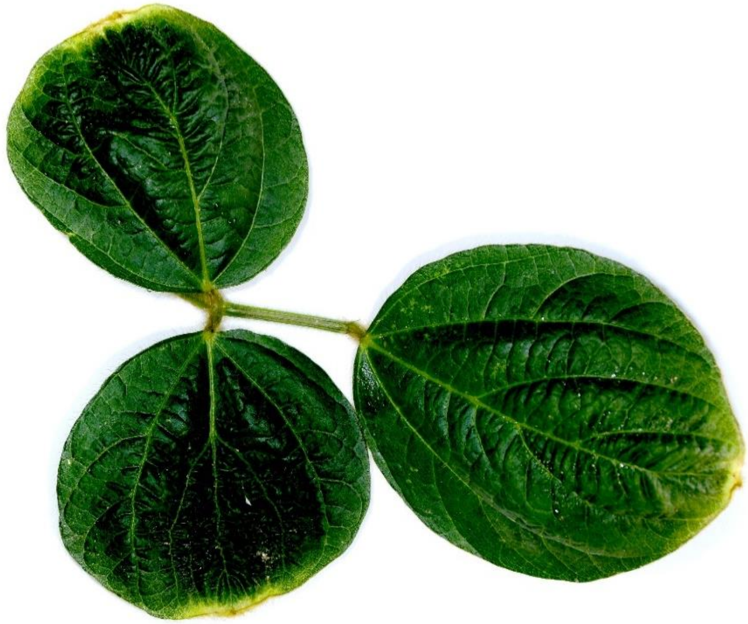

Figure 10. Trifoliate image 21 days after treatment dicamba at 0.01X rate.

Figure 11. Trifoliate image 21 days after treatment dicamba at 0.1X rate.

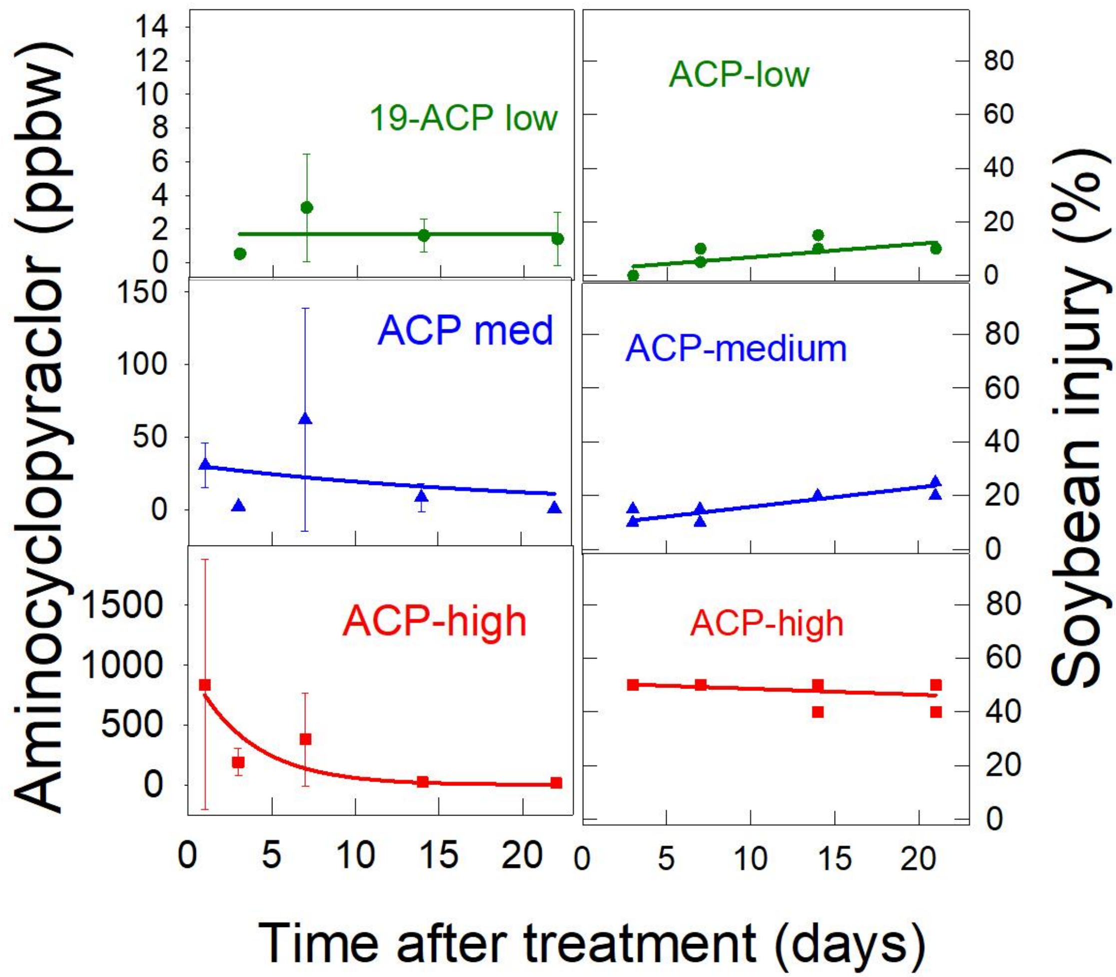

Figure 12. Aminocyclopyrachlor (ACP) concentrations in soybean leaf samples and soybean injury in 2019.

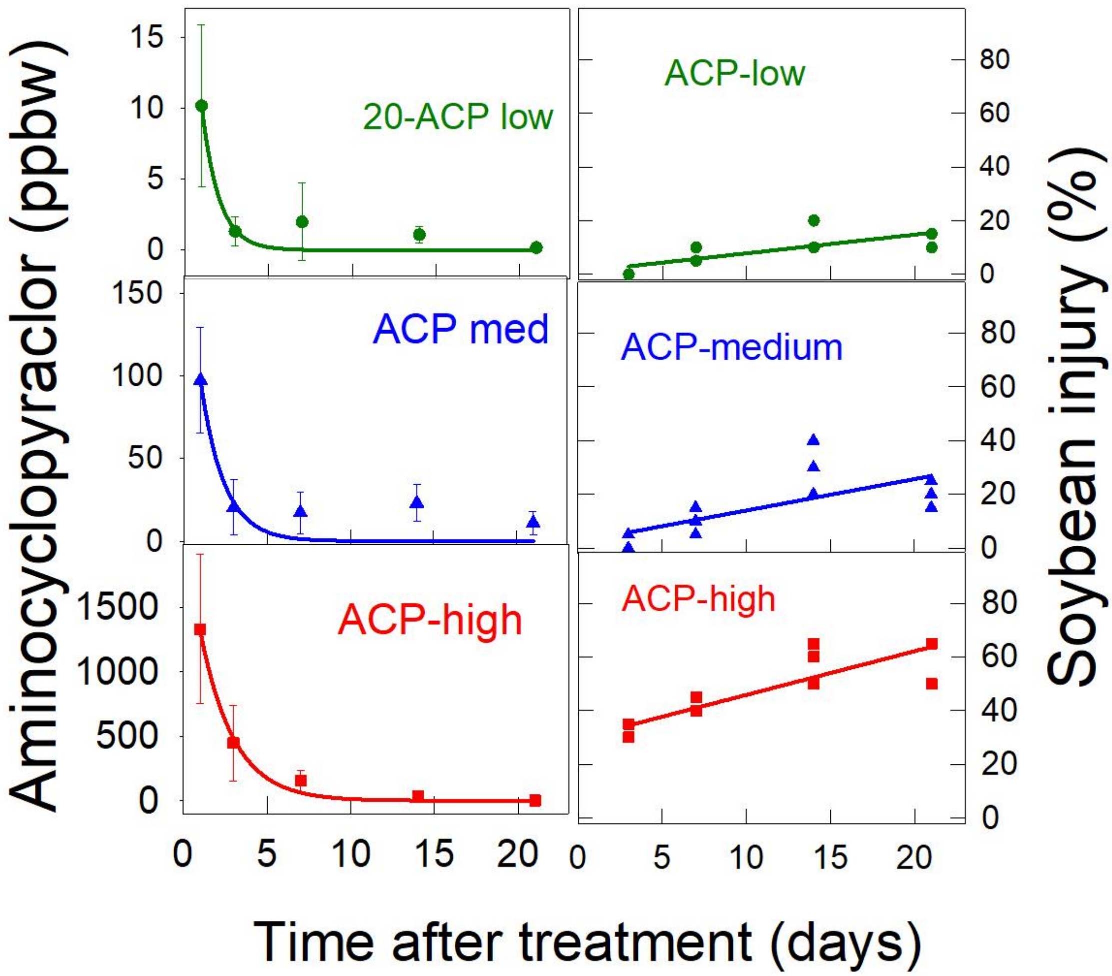

Figure 13. Aminocyclopyrachlor (ACP) concentrations in soybean leaf samples and soybean injury in 2020.

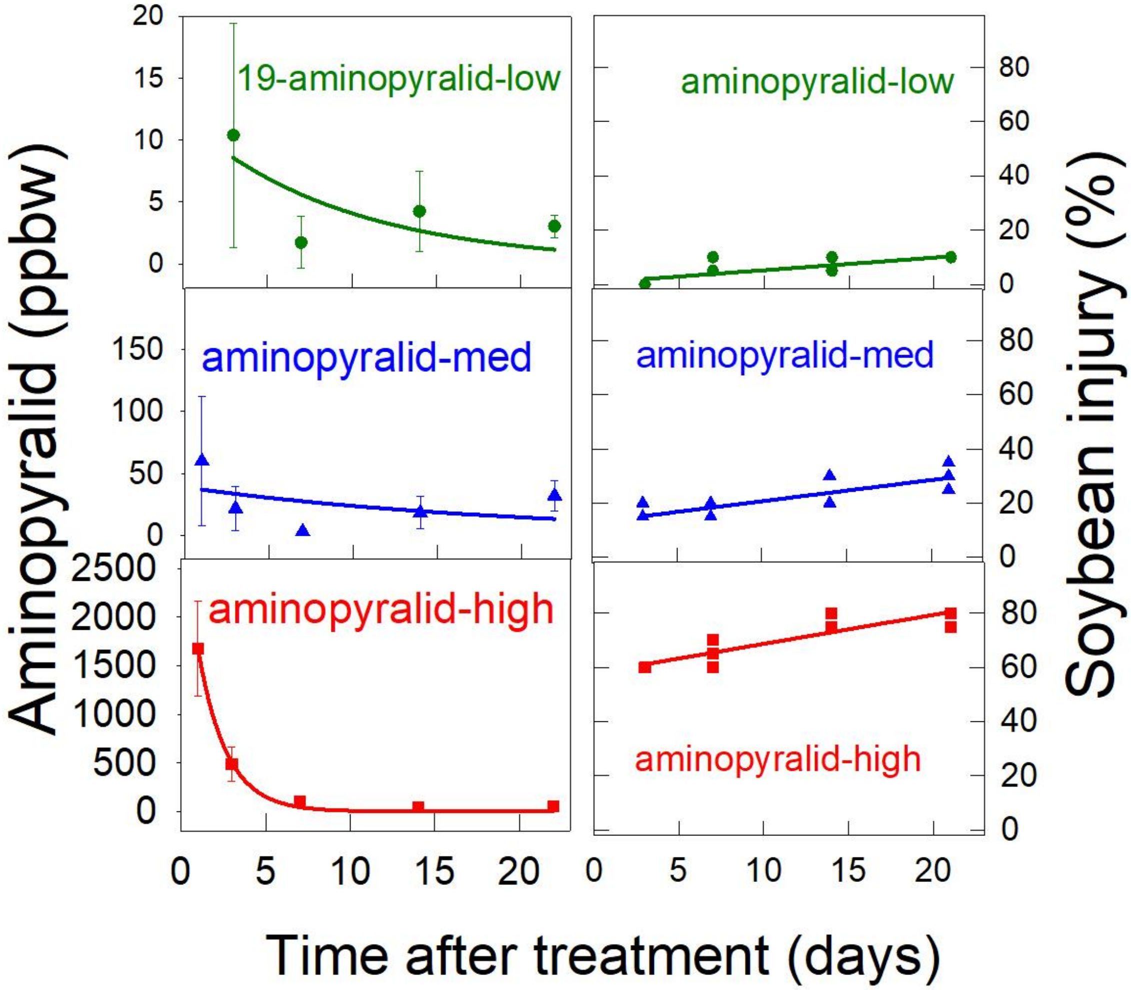

Figure 14. Aminopyralid concentrations in soybean leaf samples and soybean injury in 2019.

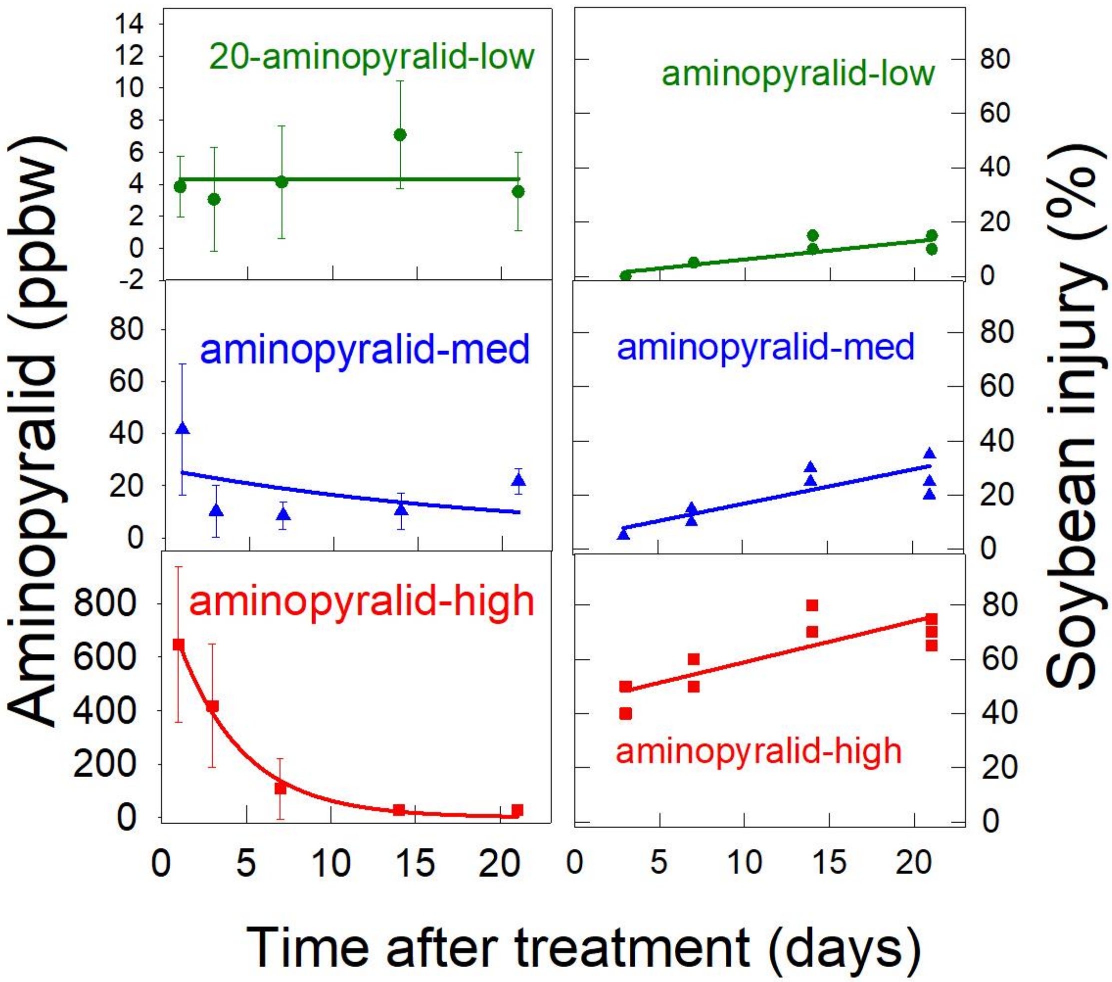

Figure 15. Aminopyralid concentrations in soybean leaf samples and soybean injury in 2020.

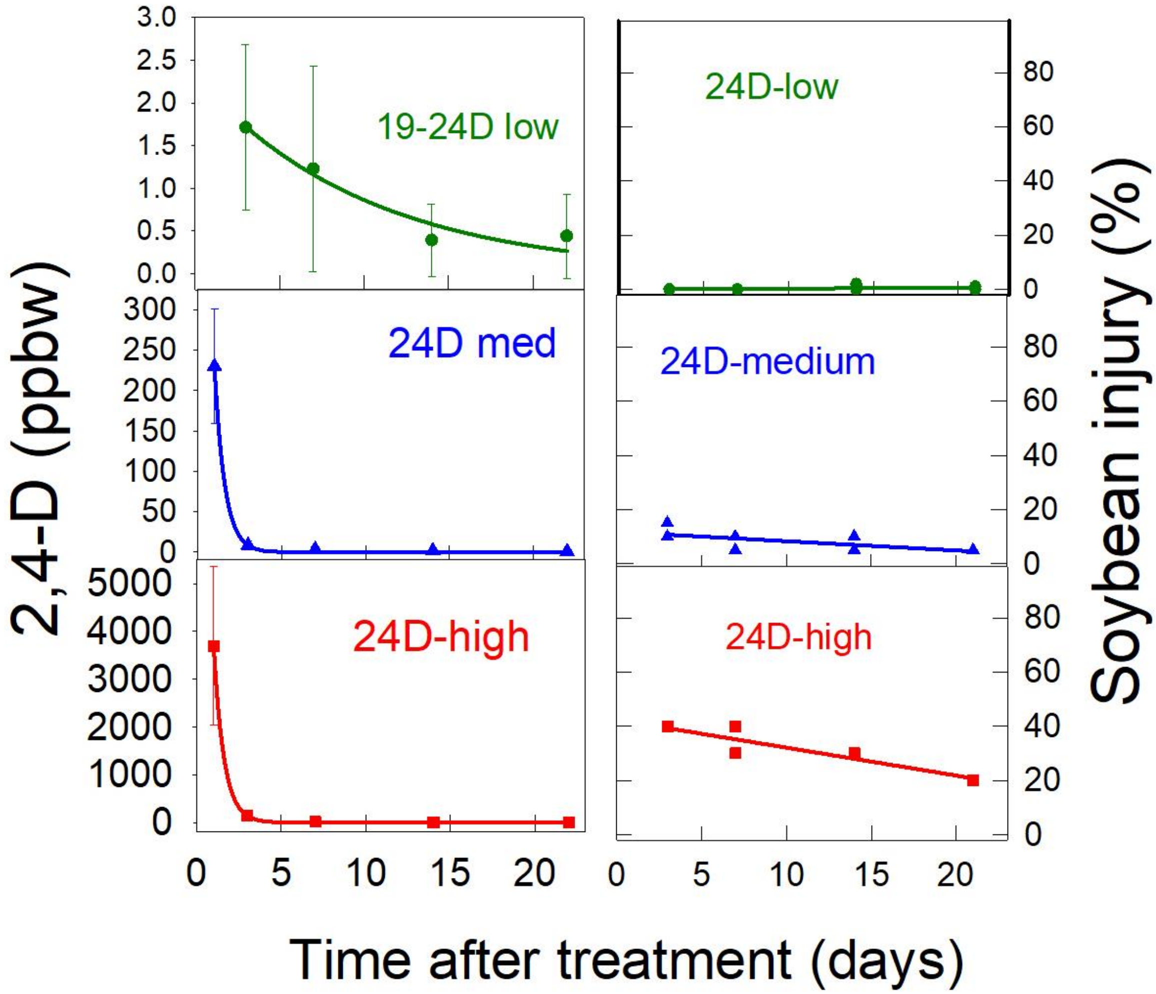

Figure 16. 2,4-D concentrations in soybean leaf samples and soybean injury in 2019.

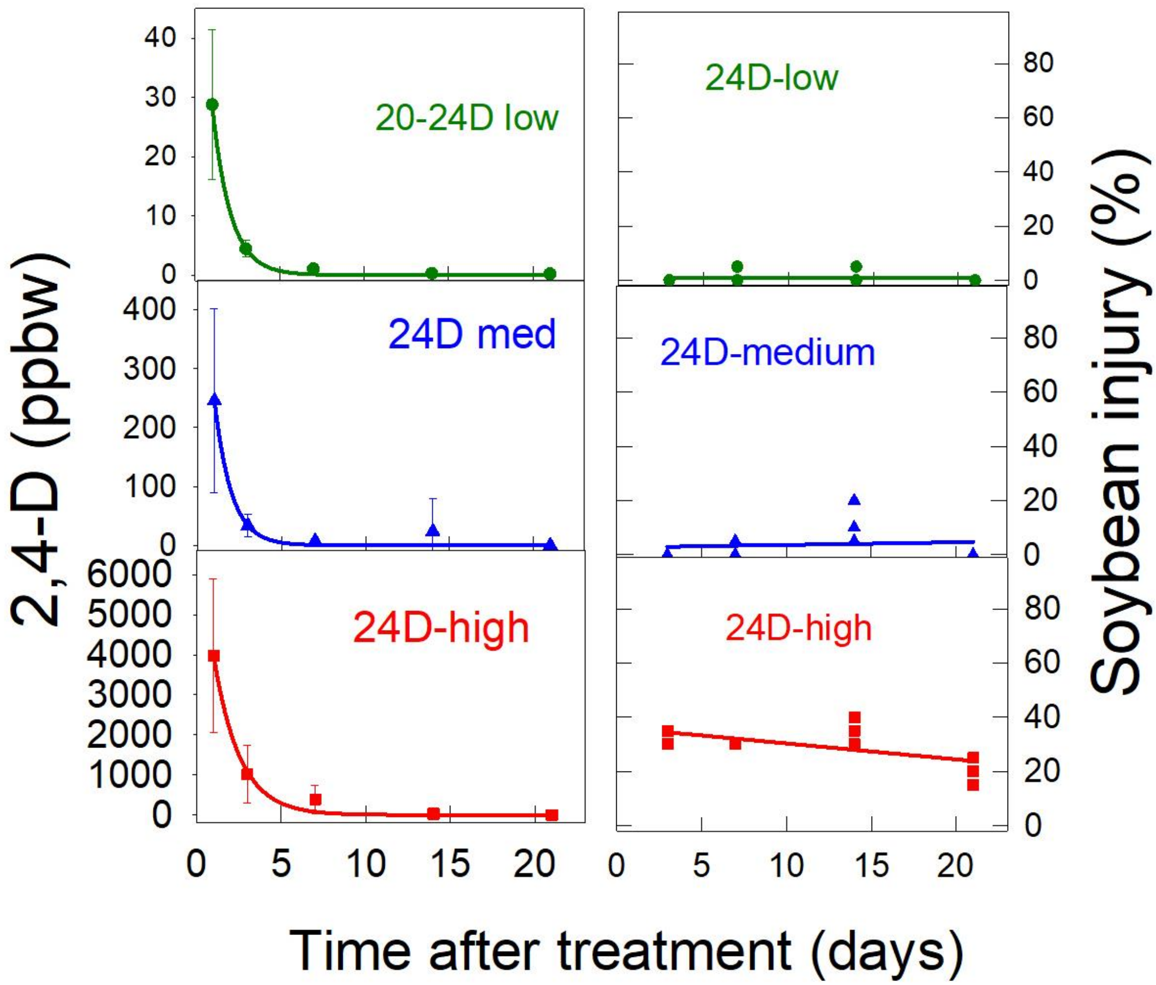

Figure 17. 2,4-D concentrations in soybean leaf samples and soybean injury in 2020.

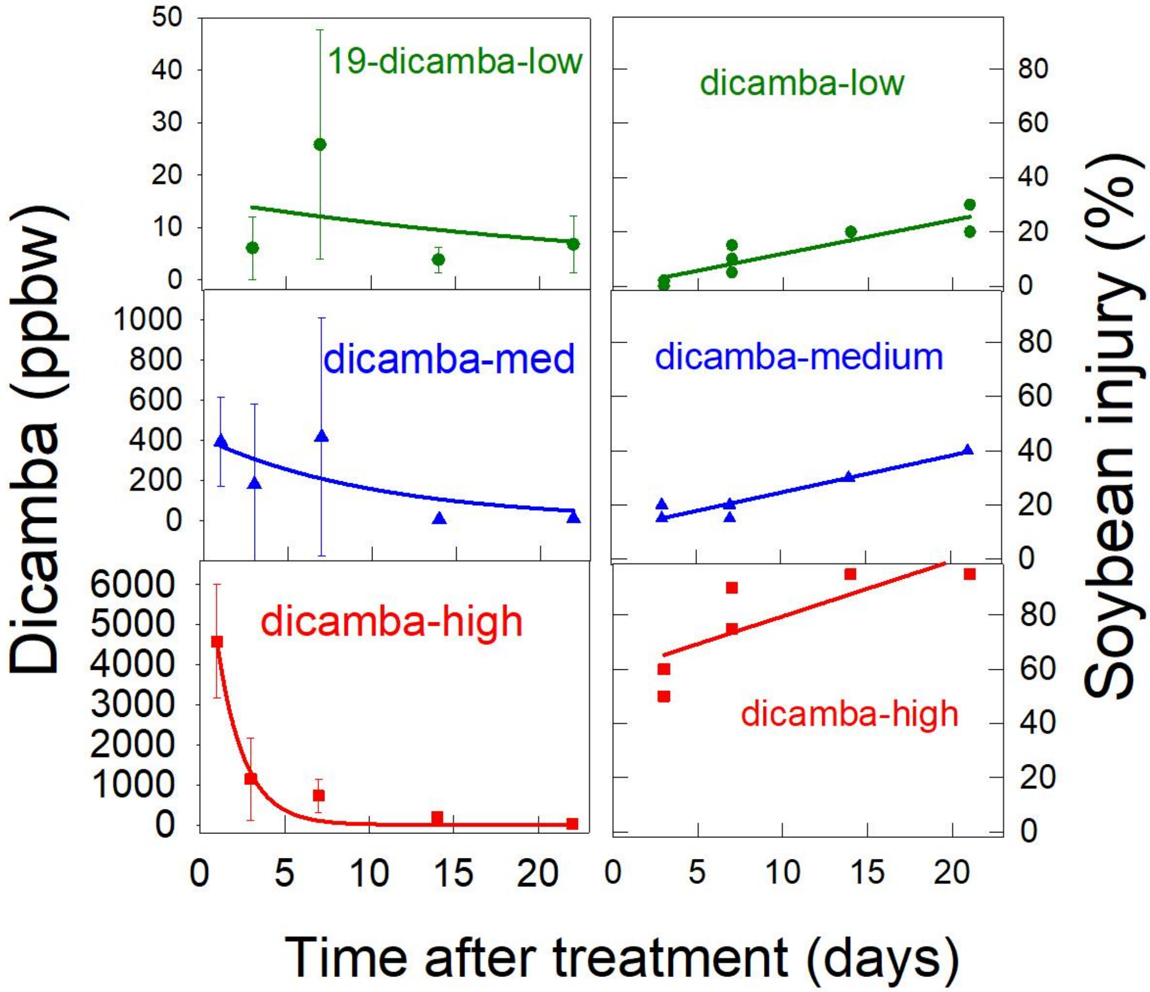

Figure 18. Dicamba concentrations in soybean leaf samples and soybean injury in 2019.

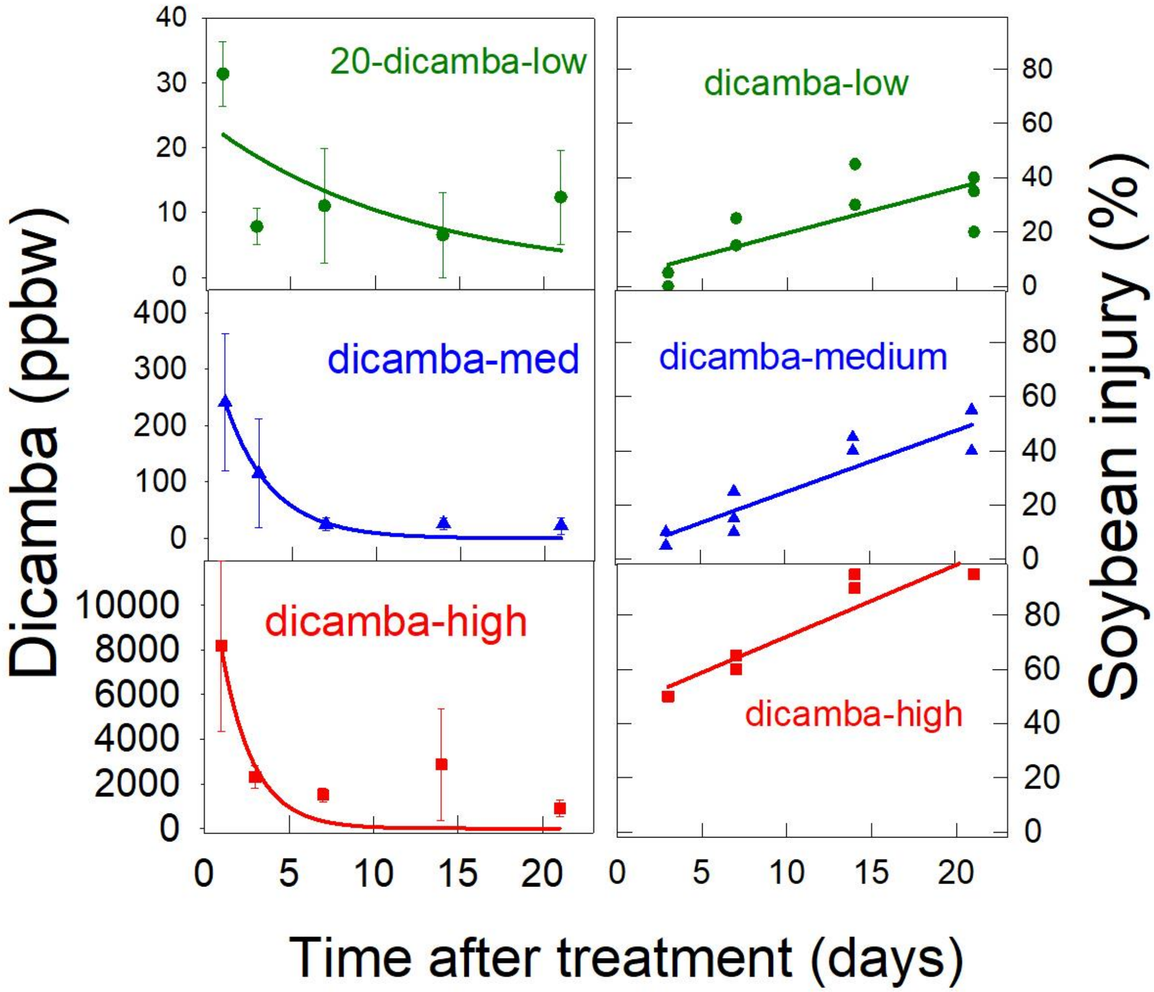

Figure 19. Dicamba concentrations in soybean leaf samples and soybean injury in 2020.


Figure 20. Aminocyclopyrachlor applied as a PRE at 1/2X rate.


Figure 21. Aminopyralid applied as a PRE at 1/2X rate.

Figure 22. Dicamba applied as a PRE at 1/2X rate.

Figure 23. 2,4-D applied as a PRE at 1/2X rate.

Vita

Trey I. Clark is a native of Greeneville, Tennessee. He graduated with the class of 2013 from Greeneville High School. He achieved an Associates of Science in Agriculture from Walters State Community College in 2016. After Walters State, he transferred to the University of Tennessee, Knoxville where he earned his Bachelor of Science in Agriculture with a concentration in Agriculture Extension Education in 2018. In 2019, he accepted a position as a Research Technician III and began his Master of Science degree in Plant Science at the University of Tennessee. In 2021 he completed his Master of Science degree in Plant Science at the University of Tennessee. He is very blessed and thankful for the support from his family, friends, and co-workers.