Weed Technology

www.cambridge.org/wet

Assessment of dicamba and 2,4-D residues in Palmer amaranth and soybean

Maria Leticia Zaccaro-Gruener[1], Jason K. Norsworthy[2], Leonard B. Piveta[3], L. Tom Barber[4] and Andy Mauromoustakos[5]

[1]Graduate Research Assistant, University of Arkansas System Division of Agriculture, Fayetteville, AR, USA; [2]Distinguished Professor and Elms Farming Chair of Weed Science, University of Arkansas System Division of Agriculture, Fayetteville, AR, USA; [3]Research Scientist, University of Arkansas System Division of Agriculture, Fayetteville, AR, USA; [4]Professor and Extension Weed Scientist, University of Arkansas System Division of Agriculture, Lonoke, AR, USA and [5]Professor, Agricultural Statistics Laboratory, University of Arkansas, Fayetteville, AR, USA

Research Article

Cite this article: Zaccaro-Gruener ML, Norsworthy JK, Piveta LB, Barber LT, Mauromoustakos A (2023) Assessment of dicamba and 2,4-D residues in Palmer amaranth and soybean. Weed Technol. **37**: 431–444. doi: 10.1017/wet.2023.60

Received: 17 May 2023
Revised: 7 August 2023
Accepted: 17 August 2023
First published online: 4 September 2023

Associate Editor:
Prashant Jha, Iowa State University

Nomenclature:
2,4-D; dicamba; Palmer amaranth; *Amaranthus palmeri* S. Watson; soybean; *Glycine max* (L.) Merr.

Keywords:
Auxin off-target movement; plant tissue analysis; auxin injury

Corresponding author:
Maria Leticia Zaccaro-Gruener;
Email: mzaccaro@uark.edu

Abstract

Off-target movement of 2,4-D and dicamba is sometimes to blame as the cause of symptoms observed in weeds growing in production fields. Pesticide regulatory authorities routinely sample tissues of weeds or crops from fields under investigation for potential illegal use of auxin herbicides. This research aimed to determine if analytical tests of herbicide residue on soybean or Palmer amaranth vegetation treated with dicamba or 2,4-D could be used to differentiate between rates applied and how the residue levels decay over a 1-mo interval. Four rates of each herbicide (1X, 0.1X, 0.01X, and 0.001X) were applied, with a 1X rate of dicamba and 2,4-D assumed to be 560 and 1,065 g ae ha^{-1}, respectively. Experiments included dicamba- and 2,4-D-resistant soybean (Xtend® and Enlist® traits, respectively) and Palmer amaranth categorized by size (8 to 15 cm, 20 to 30 cm, and 35 to 50 cm in height). Analytical results show that herbicide residues were detected above detection limits of 0.04 μg g^{-1} for dicamba and 0.004 μg g^{-1} for 2,4-D, respectively, particularly for samples treated with a 1X and 0.1X rate of dicamba or 2,4-D. Nondetections were frequent, even as early as 2 to 3 d after treatment (DAT), with 0.01X and 0.001X rates of 2,4-D or dicamba. Residues declined rapidly on Xtend® soybean treated with dicamba and on Enlist® soybean treated with 2,4-D. The severity of auxin symptomology generally agreed with the ability to detect dicamba or 2,4-D residue in plant tissue for Palmer amaranth, whereas for soybean, this was not always the case. Hence detecting dicamba or 2,4-D residues in both Palmer amaranth and soybean vegetation, along with visible symptoms on both plants during investigations, would generally indicate an earlier direct application of the auxin herbicide rather than off-target movement being the cause of detection.

Introduction

The adoption of 2,4-D- and dicamba-resistant technology, particularly for soybean production, increased the use of these herbicides during the summer months in the growing season (Arneson and Werle 2020; Werle et al. 2018). The commercial introduction of the dicamba-resistant trait (Xtend®) by Bayer Crop Science (St. Louis, MO, USA) in 2016 (Wechsler 2018) and the 2,4-D-resistant trait (Enlist®) by Corteva Agriscience (Indianapolis, IN, USA) in 2019 (Schmidt 2019) allowed the use of new dicamba and 2,4-D formulations, respectively, for in-crop postemergence weed control in soybean in the United States. Previous research showed that weed management programs that included 2,4-D and dicamba were effective in controlling Palmer amaranth and waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], which are the most troublesome weeds in soybean production in the United States (Cahoon et al. 2015; Meyer et al. 2015; Spaunhorst and Johnson 2017; Van Wychen 2022).

Dicamba and 2,4-D are synthetic auxin herbicides (WSSA Site of Action Group 4) classified as benzoic acid and phenoxy acid, respectively (Shaner 2014). The herbicidal activity of synthetic auxins is a result of the impact on natural auxin receptors that regulate physiological and metabolic activities, further resulting in abnormal cell expansion and division (Grossmann 2010), subsequently causing epinasty, leaf cupping, stem twisting, callus tissue, stunting, and necrosis (Zimdahl 2013). One of the main mechanisms making dicotyledonous species susceptible to synthetic auxins is the metabolization of these compounds to inactive compounds that can readily be converted to the parent acid phytotoxic form, while tolerant species process these into irreversible inactive compounds (Peterson et al. 2016). Meanwhile, dicamba-resistant crops received the dicamba monooxygenase gene from bacteria that promote dicamba metabolism to 3,6-dichlorosalicyclic acid (DCSA) by the Rieske nonheme monooxygenase enzyme (Behrens et al. 2007). Resistance to 2,4-D was engineered in crops by incorporating

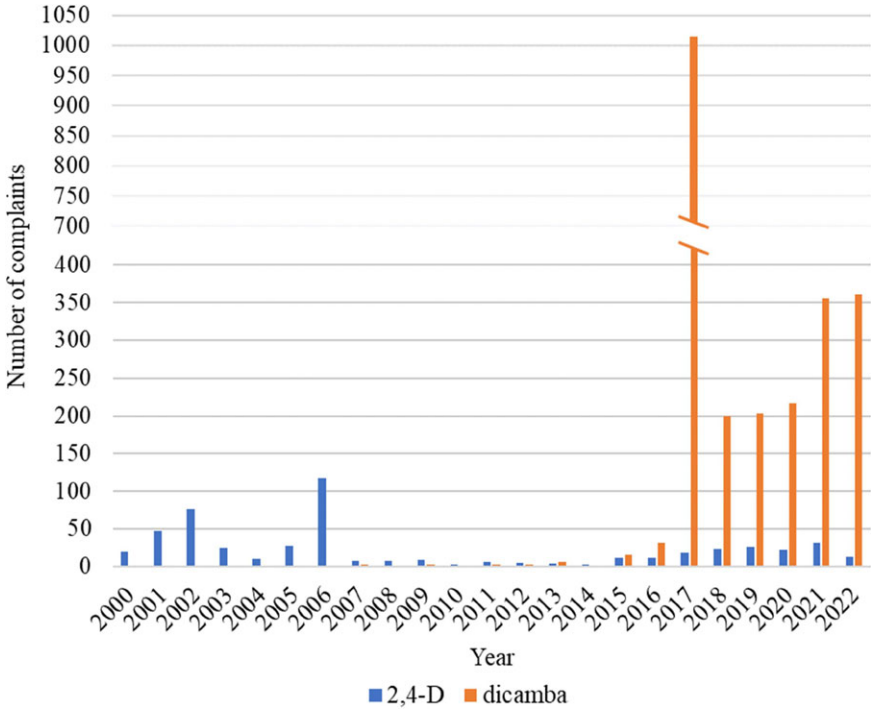

Figure 1. The total number of official complaints filed with the Arkansas State Plant Board of alleged plant damage related to the off-target movement of dicamba or 2,4-D in Arkansas from 2000 to 2022. Data are from the Arkansas Department of Agriculture, Pesticide Division, 2022.

genes that allow for the metabolism of 2,4-D into dichlorophenol by the aryloxyalkanoate dioxygenase enzyme (Wright et al. 2010). Surprisingly, considering that these herbicides have been commercially applied since the 1940s and 1960s, respectively (Timmons 1970; Troyer 2001), only 17 studies have reported weed species resistant to 2,4-D and dicamba in the United States (Heap 2023).

Previous studies have shown that 2,4-D and dicamba are prone to off-target movement by draftable spray particles and volatility (Akesson and Yates 1964; Behrens and Lueschen 1979; Bish et al. 2021; Jones et al. 2019; Maybank et al. 1978; Mueller et al. 2013; Soltani et al. 2020; Sosnoskie et al. 2015; Werle et al. 2022). Hence the agrochemical industry invested in formulations of 2,4-D and dicamba to reduce the off-target movement of the herbicides. Currently only the choline salt of 2,4-D, which is present in Enlist One® and Enlist Duo® formulations (Anonymous 2022b, 2022c), is labeled for over-the-top application in 2,4-D-resistant crops, while the N,N-Bis-(3-aminopropyl)methylamine salt of Engenia® (BASF, Research Triangle Park, NC, USA) (Anonymous 2022a) and the diglycolamine salt of dicamba contained in Tavium® (Syngenta Crop Protection, Greensboro, NC, USA) and XtendiMax® (Bayer Crop Science, St. Louis, MO, USA) (Anonymous 2022d, 2022e) are allowed for dicamba-resistant technologies. According to survey results, nearly half of the soybean planted in 2017 in Tennessee, Missouri, Mississippi, and Arkansas was dicamba-resistant; the later-released 2,4-D-resistant varieties were expected to show similar success (Steckel et al. 2017).

Nevertheless, reports of auxin damage to sensitive vegetation, particularly concerning dicamba, have occurred since initial uses (Auch and Arnold 1978) and have increased exponentially following the introduction of dicamba-resistant technologies (Bradley 2018, 2017; Hartzler and Jha 2020; Steckel 2018, 2019). According to data provided by the Arkansas Department of Agriculture (S. Nichols, unpublished data, 2022) regarding the total number of complaints related to alleged plant damage attributed to 2,4-D and dicamba, the number of 2,4-D cases reported from 2000 to 2022 remained fewer than 50 per year, except for 2002 and 2006, which had 76 and 118 cases reported, respectively (Figure 1). The number of reported cases attributed to dicamba damage was fewer than 6 per year from 2000 to 2014, but after 2015, a significant increase occurred, with a peak in 2017, during which 1,014 complaints were reported (Figure 1). The peak in complaints occurred one year after the introduction of dicamba-resistant soybean.

Investigations conducted by the state pesticide regulatory agencies after official complaints consist of documentation of the damage and a collection of impacted plant tissue for analysis of herbicide content (S. Nichols, personal communication, 2022). However, because negative results of herbicide presence are common, pesticide regulatory authorities have questioned the success of plant tissue analysis in off-target movement inquiries. Therefore the objectives of this research were (1) to determine the residue amount of 2,4-D and dicamba present on Palmer amaranth and soybean over a 1-mo period following treatments simulating a direct application and drift rates of herbicides and (2) to evaluate the impact of soybean technology (dicamba or 2,4-D resistance traits) on in-plant detection of auxin herbicide residues.

Material and Methods

Detection of Dicamba and 2,4-D in Palmer Amaranth

An experiment was conducted at the Milo J. Shult Agricultural Research and Extension Center of the University of Arkansas, near Fayetteville, AR (36.1°N, 94.18°W), in 2019 and 2020. A field with a native population of Palmer amaranth was divided into three blocks, each with eight plots measuring 4 × 25 m. The soil classification in this field was a Leaf silt loam (18% sand, 69% silt,

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

and 13% clay, with 1.6% organic matter and pH 6.6). On the day of treatment, Palmer amaranth plants in each plot were classified and labeled according to small, medium, and large size, corresponding to 8 to 15 cm, 20 to 30 cm, and 35 to 50 cm in height, respectively. Herbicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with Turbo TeeJet® induction 110015 nozzles (TeeJet® Technologies, Wheaton, IL, USA) calibrated to deliver 140 L ha^{-1} at 4.8 km h^{-1}. 2,4-D (Enlist One®) was applied at 1,065 (1X rate), 106.5 (0.1X rate), 10.65 (0.01X rate), and 1.065 (0.001X rate) g ae ha^{-1}. Dicamba (XtendiMax® with VaporGrip® technology) was applied at 560 (1X rate), 56 (0.1X rate), 5.6 (0.01X rate), and 0.056 (0.001X rate) g ae ha^{-1}. All treatments contained 0.25% v/v of nonionic surfactant. The application dates were July 12, 2019, and July 8, 2020. Tissue samples were taken by cutting plants at the soil level, placing them in labeled plastic bags, and immediately storing them at −20 C. Plant sampling was performed starting from the day of application (at least 1 h after treatment to allow dryness) (three replicates) until 29 d after treatment (DAT). A total of 8 and 10 collections occurred in 2019 and 2020, respectively. The collection of plants was done at midday to avoid the presence of dew on leaves. Weather conditions, including air temperature and precipitation, were monitored during these experiments using a weather station approximately 100 m from the test site.

Herbicide residue analysis was executed at the analytical laboratory of the Arkansas Department of Agriculture in Little Rock, AR, the same lab used for all investigative samples collected by the Arkansas State Plant Board. The method for herbicide extraction was modified from Andersen et al. (2004). Plant tissue was homogenized, and a 3- to 5-g aliquot was dissolved in 40 mL of 0.1 M NaOH and filtered. The filtrate was transferred to another bottle and dissolved using 10 mL of 0.33 M H_2SO_4 with 10 mL of ethyl-acetate for extraction. After centrifugation at 4,000 rpm for 5 min, the organic layer was collected and taken to dryness. Then, the sample was resuspended to 1 mL volume. The sample was sonicated, filtered, evaporated, and exchanged to solvent with 75% acetonitrile in water plus 0.1% formic acid. Quality control tests consisted of blank matrix samples (plant tissue) without herbicide and fortified matrix samples, including internal standards using 0.01 to 0.11 µg g^{-1} for dicamba and 0.001 to 0.04 µg g^{-1} for 2,4-D. Calibration curves were defined using best-fit regression with $R^2 \geq 0.995$ and no interfering peaks from impurities.

The method for herbicide quantification was adapted from Chamkasem and Morris (2016). Herbicide quantification was done by high-performance liquid chromatography (Agilent 1290; Agilent, Santa Clara, CA, USA) paired with a quadrupole-quadrupole time-of-flight mass spectrometer (X500R QTOF; AB Sciex, Framingham, MA, USA). Liquid chromatography was performed using a Kinetex C18 column (2.6 µm, 100 Å, 100 × 4.6 mm; Phenomenex, Torrance, CA, USA). The mobile phase comprised 0.1% formic acid in water and acetonitrile with 0.1% formic acid as the organic solvent. The constant flow rate was 0.8 mL min^{-1}, and the gradient program was 0 to 1 min of 5% B and 1 to 5 min of 95% B. Spectrometer detection of negatively charged ions was achieved with multiple-reaction monitoring mode, a set capillary voltage of 2.50 kV, and a temperature of 600 C. Quantification of dicamba was based on the area of fragment ion 174.9728 Da, whereas 2,4-D had fragment ion equal to 160.9564 Da (retention time equal to 4.77 min for dicamba and 5.04 min for 2,4-D). The method limit of quantification for herbicide analysis was determined to be at 0.004 µg g^{-1} of 2,4-D and 0.04 µg g^{-1} of dicamba. Only the parent herbicidal compounds of 2,4-D and dicamba, not metabolites, were considered for these experiments.

Detection of 2,4-D and Dicamba in Soybean

Two experiments were conducted in fields at the Milo J. Shult Agricultural Research and Extension Center of the University of Arkansas in 2020 and 2021. The soil classifications of the fields were, respectively, Leaf silt loam (11% sand, 77% silt, and 12% clay, with 1.4% organic matter and pH 6.7) and Captina silt loam (22% sand, 60% silt, and 18% clay, with 1.18% organic matter and pH 6.4). Four-row plots (4 × 6 m) were established, where two rows were planted with a dicamba-resistant soybean (Xtend®; 'AG 47X6 RR2X', Asgrow Seed, Creve Coeur, MO, USA) and the others had 2,4-D-resistant soybean (Enlist®; 'P 48T22E', Pioneer Hi-Bred, Des Moines, IA, USA). Both cultivars were planted at a rate of 360,000 seeds ha^{-1} on May 22, 2020, and May 11, 2021. These fields were managed according to the recommendations of the University of Arkansas System Division of Agriculture. Air temperature and rainfall were monitored throughout the experiment using the previously mentioned weather station.

Herbicide treatments were made utilizing a CO_2-pressurized backpack sprayer equipped with Turbo TeeJet® induction 110015 nozzles calibrated to deliver 140 L ha^{-1} at 4.8 km h^{-1}. Dicamba (XtendiMax® with VaporGrip® technology) was applied at 560 (1X rate), 56 (0.1X rate), 5.6 (0.01X rate), and 0.056 (0.001X rate) g ae ha^{-1}. 2,4-D (Enlist One®) was treated at 1,065 (1X rate), 106.5 (0.1X rate), 10.65 (0.01X rate), and 1.065 (0.001X rate) g ae ha^{-1}. All treatments included 0.25% v/v of nonionic surfactant. The application dates were July 8, 2020, and June 29, 2021. Treatments had four replications. Plant tissue samples were taken from both cultivars by cutting them at the soil level, placing them in separate identified plastic bags, and immediately storing them at −20 C. Plant sampling was done randomly, starting from the day of treatment (no less than 1 h after treatment) until 31 DAT. A total of 10 collections occurred in both years. Plots were sampled from the lowest to the highest concentration of each herbicide. Herbicide residue analysis was executed at the analytical laboratory of the Arkansas Department of Agriculture using the previously mentioned method.

Statistical Analyses

Herbicide residue (µg g^{-1}) data were evaluated using the univariate procedure in JMP Pro 17 (SAS Institute Inc., Cary, NC). Given the objectives of each experiment, data analysis was conducted separately for each herbicide. For the Palmer amaranth experiment, tests considered the impact of year, herbicide rate, average plant size at treatment, and sampling date. Dicamba or 2,4-D residue data, assuming lognormal distribution, were analyzed using generalized regression in the Fit Model platform of JMP Pro 17, with Lasso estimation, and were validated using the corrected Akaike's information criterion. For the soybean experiment, year, soybean trait, herbicide rate, and sampling time were analyzed using a similar generalized regression procedure to that described earlier. Regression analyses were censored using the lower detection limit associated with each compound analyzed: 0.04 µg g^{-1} for dicamba and 0.004 µg g^{-1} for 2,4-D (SAS Institute Inc. 2022). The generalized R^2 value generated indicated the predictive power of a nonlinear model (Nagelkerke 1991), which was strongest when the value was closest to 1. Analysis of inverse predictions with respect to the number of days in which herbicide detections were above analytical limits was conducted when appropriate.

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

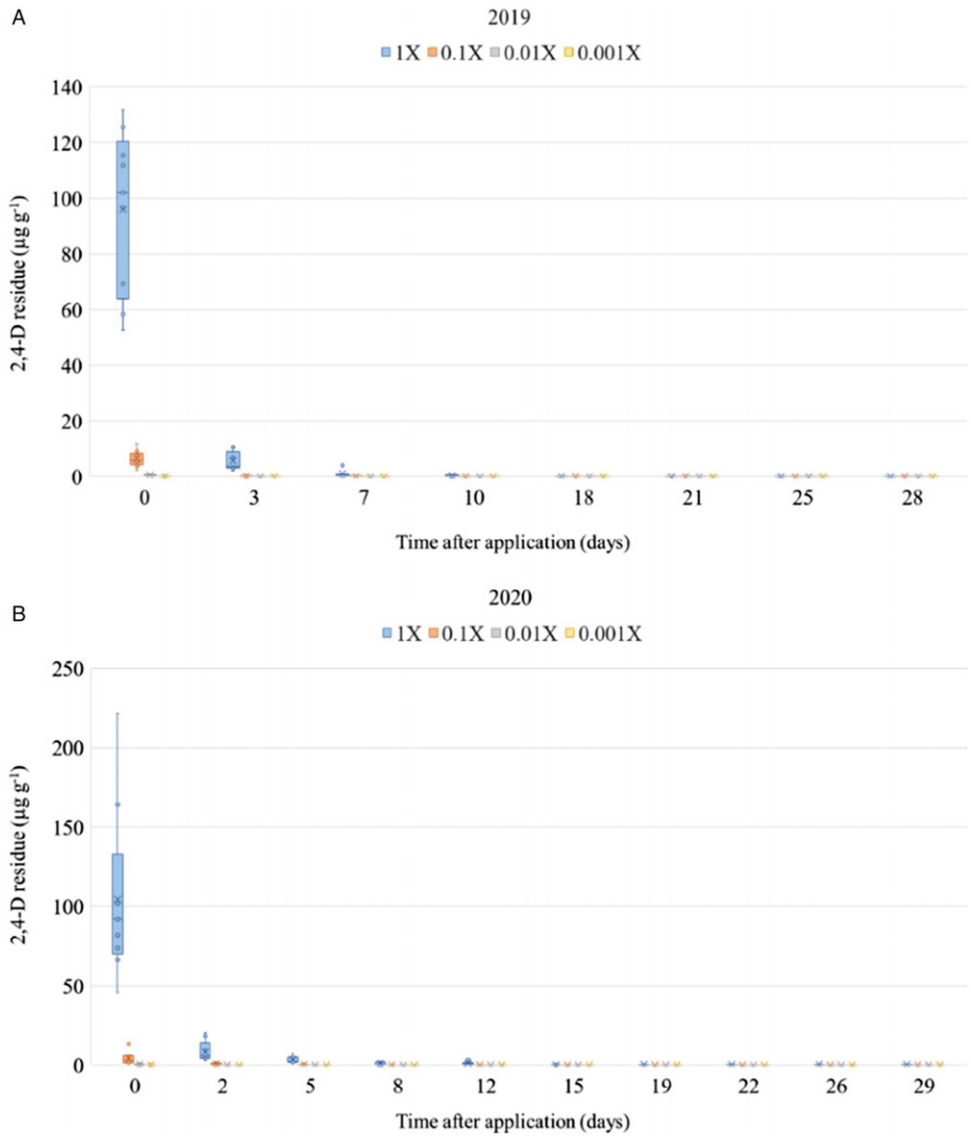

Figure 2. Distribution of 2,4-D residue (µg g^{-1}) detected in Palmer amaranth over time after treatment with 2,4-D at 1X, 0.1X, 0.01X, and 0.001X rates (blue, orange, gray, and yellow boxes, respectively), with 1X being 1,065 g ae ha^{-1}, averaged over plant size at the application in 2019 (A) and 2020 (B). 2,4-D residue in 2019 regressed as a function of time after application using the equations $Y_{1X} = \exp(4.52 - 0.33X)$ (generalized $R^2 = 0.74$), $Y_{0.1X} = \exp(2.11 - 0.73X)$ (generalized $R^2 = 0.83$), and $Y_{0.01X} = \exp(-0.46 - 1.55X)$ (generalized $R^2 = 0.77$); the relationship for the 0.001X treatment was not significant. 2,4-D residue in 2020 regressed as a function of time after application using the equations $Y_{1X} = \exp(4.66 - 0.24X)$ (generalized $R^2 = 0.55$), $Y_{0.1X} = \exp(1.79 - 0.33X)$ (generalized $R^2 = 0.67$), $Y_{0.01X} = \exp(-0.16 - 0.98X)$ (generalized $R^2 = 0.71$), and $Y_{0.001X} = \exp(-3.03 - 0.60X)$ (generalized $R^2 = 0.64$).

Results and Discussion

The laboratory extraction and analysis of Palmar amaranth or soybean vegetative tissue collected in the plots treated with 2,4-D and dicamba successfully detected these herbicides in most samples. Nontreated plant samples did not result in herbicide detections.

Dicamba and 2,4-D Residues in Palmer Amaranth

The herbicide residue recovered in laboratory tests with Palmer amaranth tissue varied by year, treatment rate, and time after application. Therefore herbicide recovery data are displayed separately by herbicide treatment, year, and rate applied. Palmer amaranth size at application impacted herbicide recovery only in 2020 ($P = 0.0112$ and 0.0002, respectively; data not shown). However, differences could be expected due to the amount of herbicide intercepted by plants with larger canopies versus smaller ones, influencing detection results. Additionally, younger and smaller plants tend to have thinner leaf cuticles, which allows for greater herbicide uptake (Zimdahl 2013). Therefore data were averaged over plant size to simplify and generalize interpretations (nine observations).

The concentration of herbicides recovered in Palmer amaranth tissue declined rapidly after application. Following a 1X rate of dicamba or 2,4-D, the respective herbicide was detected in six of nine Palmer amaranth samples, even at 28 or 29 DAT. Palmer amaranth samples treated with a 0.1X rate of dicamba had residues detected until 10 to 15 DAT, whereas 2,4-D at the same 0.1X rate was detected only 7 to 12 DAT. When considering analytical detection results for herbicides applied at 0.01X or 0.001X of dicamba, no herbicide was detected at 10 to 12 DAT or 5 to 7 DAT, respectively. Similarly, treatment of Palmer amaranth with a 0.01X or 0.001X rate of 2,4-D led to no detection of the herbicide beyond 10 or 5 DAT, respectively (Supplementary Tables S1 and S2).

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

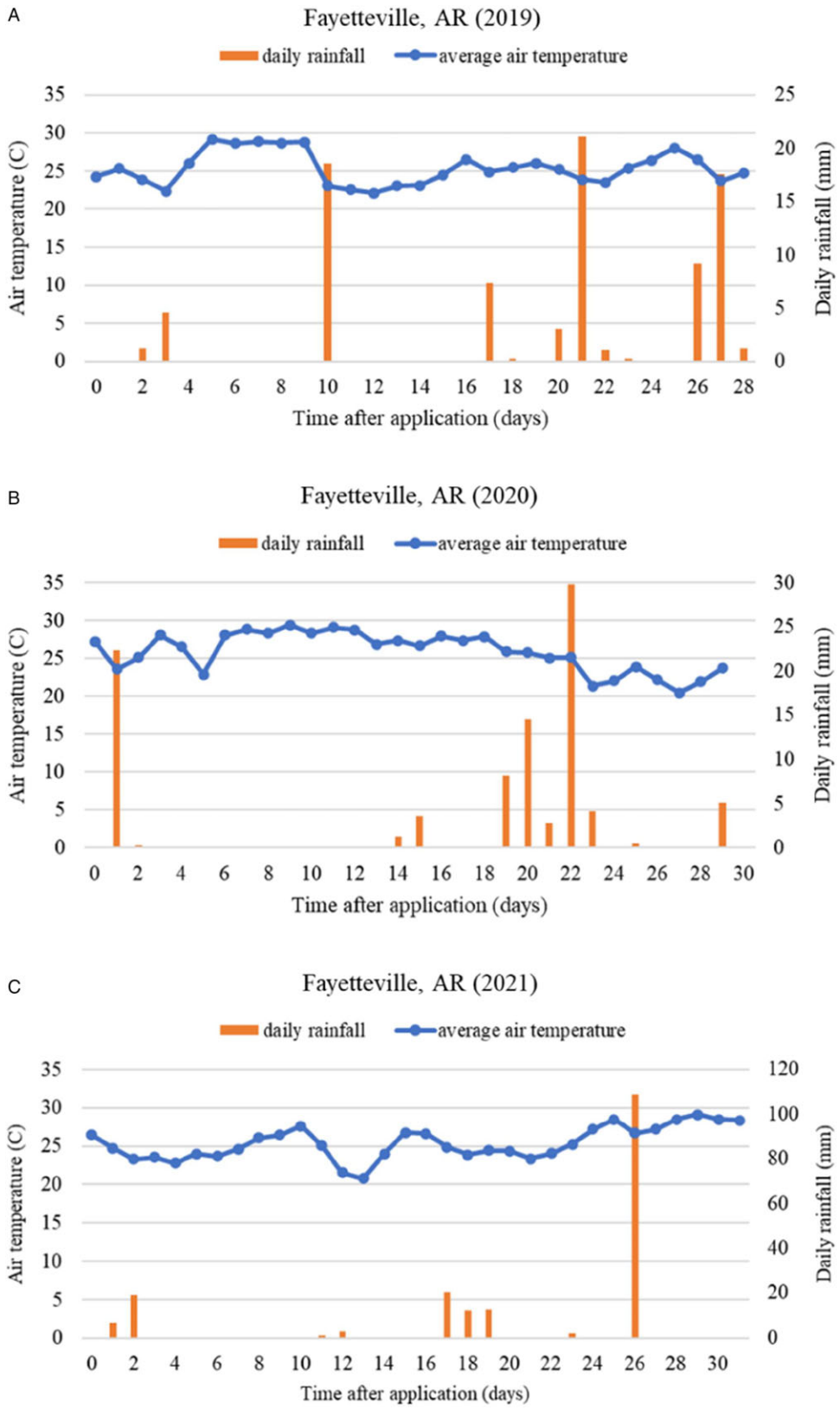

Figure 3. Daily results of observed average air temperature (C) and accumulated rainfall (mm) from the application until the day of the last collection of Palmer amaranth or soybean tissue samples made in Fayetteville, AR, from 2019 to 2021.

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

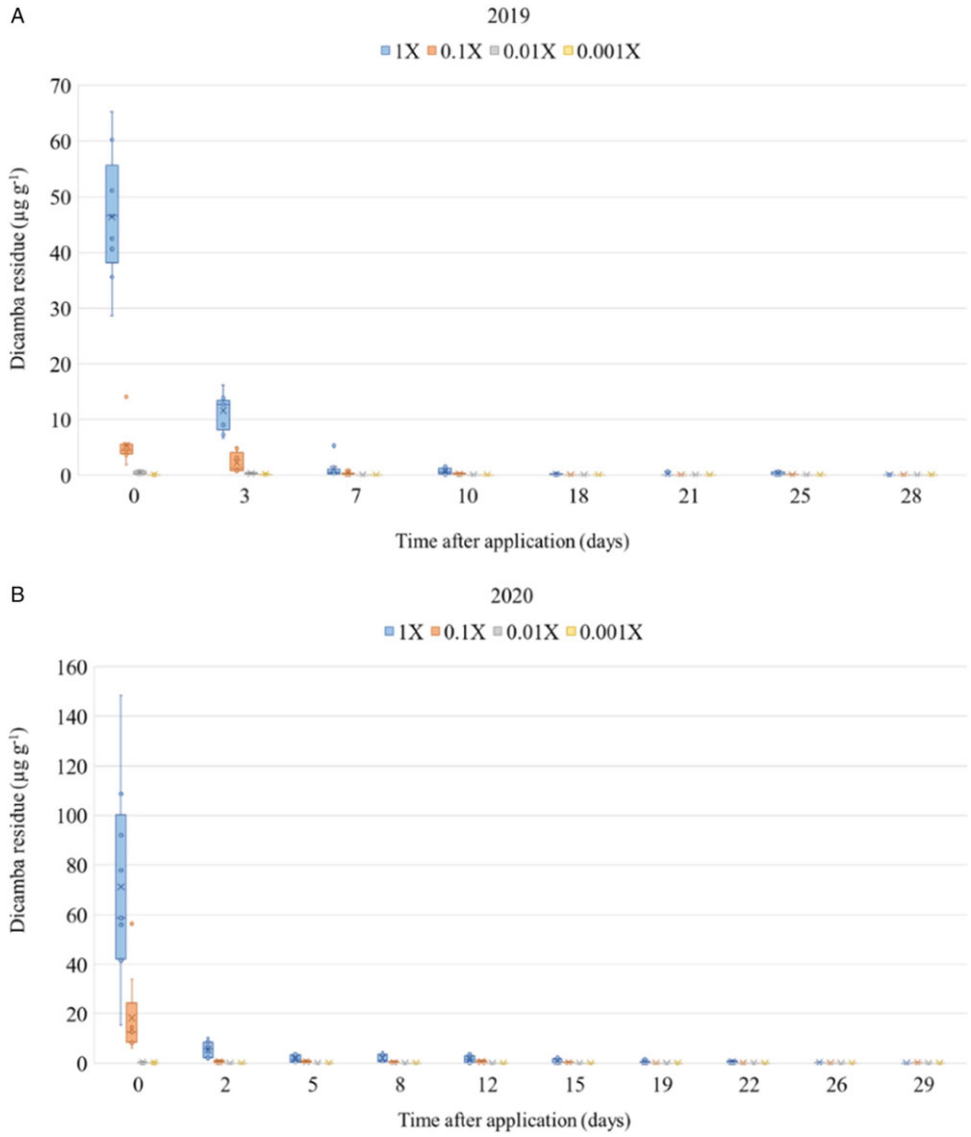

Figure 4. Distribution of dicamba residue (µg g^{-1}) in Palmer amaranth detected over time after treatment with dicamba at 1X, 0.1X, 0.01X, and 0.001X rates (blue, orange, gray, and yellow boxes, respectively), with 1X being 560 g ae ha^{-1}, averaged over plant size at the application in 2019 (A) and 2020 (B). Dicamba residue in 2019 regressed as a function of time after application using the equations $Y_{1X} = \exp(4.16 - 0.26X)$ (generalized $R^2 = 0.62$), $Y_{0.1X} = \exp(1.61 - 0.22X)$ (generalized $R^2 = 0.67$), and $Y_{0.01X} = \exp(-0.45 - 0.41X)$ (generalized $R^2 = 0.72$); the relationship for the 0.001X treatment was not significant. Dicamba residue in 2020 regressed as a function of time after application using the equations $Y_{1X} = \exp(3.35 - 0.18X)$ (generalized $R^2 = 0.60$), $Y_{0.1X} = \exp(1.37 - 0.12X)$ (generalized $R^2 = 0.36$), $Y_{0.01X} = \exp(-0.95 - 0.54X)$ (generalized $R^2 = 0.51$), and $Y_{0.001X} = \exp(-0.72 - 1.57X)$ (generalized $R^2 = 0.32$).

Considering the 648 samples tested for dicamba, no herbicide was found above the detection limit in 356 samples; 149 and 128 were treated with a 0.001X and 0.01X rate, respectively (Supplementary Tables S1 and S2). Laboratory analysis of 2,4-D residue showed that 370 of 648 samples resulted in no detected herbicide residue, and 135 and 127 of these samples were treated with a 0.001X and 0.01X rate of 2,4-D, respectively (Supplementary Tables S1 and S2). The lack of detection of either herbicide at low rates is attributed to the rapid breakdown of both compounds in Palmer amaranth. Dicamba acid is known to be metabolized into DCSA or a hydroxylated metabolite, while 2,4-D acid is metabolized into 2,4-dichlorophenol; meanwhile, both auxins could undergo a later glucose conjugation (Meyer et al. 2020; Peterson et al. 2016). The inability to consistently detect dicamba or 2,4-D at rates of 0.01X or lower indicates that tissue samples collected after a one-time exposure to drift rates of these herbicides should not result in detection using the described extraction and analytical techniques.

During these experiments, the air temperature consistently ranged from 20 to 29 C in 2019 and 2020, while daily rainfall varied substantially, possibly impacting detection (Figure 2 A and B). A 1.3-mm rainfall occurred in 2019 a day before the second sampling (3 DAT), while a 22-mm rainfall occurred at 2 DAT in 2020, before the second sampling. Rainfall possibly washed the leaf surface of nonabsorbed herbicides, and the warm temperatures increased metabolism, which could explain why herbicide concentrations declined rapidly to levels below the detection limit. Herbicide between 0 DAT and at the second collection (3 DAT for 2019 and 2 DAT for 2020) decayed more slowly in 2019 than in 2020; for instance, dicamba reduced, on average, 34 µg g^{-1} and 65 µg g^{-1} between the first and second samplings after treatment with the 1X rate in 2019 and 2020, respectively, and average 2,4-D reduction was 90 µg g^{-1} in 2019 and 96 µg g^{-1} in 2020 after a 1X treatment (Figures 3 and 4).

Generalized regression results analyzed separately for dicamba or 2,4-D treatment as a function of time after application differed

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

Figure 5. Palmer amaranth 5 d after treatment with dicamba at 560 g ae ha^{-1} (A), 56 g ae ha^{-1} (B), 5.6 g ae ha^{-1} (C), and 0.56 g ae ha^{-1} (D) and with 2,4-D at 1,065 g ae ha^{-1} (E), 106.5 g ae ha^{-1} (F), 10.65 g ae ha^{-1} (G), and 1.065 g ae ha^{-1} (H) in 2020.

by year and herbicide rate. Regression curves for Palmer amaranth treated with dicamba had generalized R^2 values ranging from 0.62 to 0.72 in 2019 and from 0.32 to 0.60 in 2020 (Figure 3). Overall, inverse prediction results show that dicamba-treated plants at 560 g ae ha^{-1} and 56 g ae ha^{-1} (simulating a 1X and a 0.1X treatment, respectively) resulted in detections up to 29 DAT (data not shown). Predictions resulted in detections up to 6 and 2 DAT in Palmer amaranth treated with dicamba at 5.6 g ae ha^{-1} and 0.56 g ae ha^{-1}, respectively (data not shown). Regression curves for 2,4-D-treated Palmer amaranth had generalized R^2 values ranging from 0.74 to 0.83 in 2019 and from 0.55 to 0.71 in 2020 (Figure 4).

Treatment with 2,4-D at 1.065 g ae ha^{-1} (0.001X) did not result in an appropriate regression curve in 2019 (generalized $R^2 < 0.2$). Generally, inverse prediction results show that 2,4-D-treated plants at 1,065 g ae ha^{-1} and 106.5 g ae ha^{-1} (simulating a 1X and a 0.1X treatment, respectively) resulted in detections up to 29 and 22 DAT, respectively (data not shown). Predictions resulted in detections up to 6 and 2 DAT in Palmer amaranth treated with 2,4-D at 10.65 g ae ha^{-1} and 1.065 g ae ha^{-1}, respectively (data not shown). Similarly, in other research, auxin herbicide residues were recovered from tomato (*Solanum lycopersicum* L.) plants at 7 DAT with 1.1 g ae ha^{-1} of 2,4-D or dicamba, but no herbicide was recovered by 14 DAT (Sirons et al. 1982).

In addition to plant tissue analysis, photographs of visible injury of plants treated with dicamba and 2,4-D as early as the second sampling (2 or 3 DAT, depending on the year) were taken. As time after treatment passed, the symptoms became more severe, particularly for plants that received 1X and 0.1X rate treatments (560 and 56 g ae ha^{-1} of dicamba or 1,065 and 106.5 g ae ha^{-1} of 2,4-D, respectively). For instance, photographs taken at 5 DAT in 2020 displayed Palmer amaranth plants with severe and mild symptoms of epinasty after treatment with 1X and 0.1X rates of dicamba or 2,4-D (Figure 5). However, auxin symptoms on the plants were not noticeable for the other herbicide treatments by 5 DAT, particularly for the 0.01X or 0.001X rate (Figure 5). Within a few days, plants treated with a 1X rate of dicamba or 2,4-D clearly showed stem twisting, stunting, and necrosis; for treatments with a 0.1X rate, stem and leaf petiole twisting were visible, but for Palmer amaranth plants treated with a 0.01X or 0.001X rate, no apparent symptoms were observed. Photographs taken at 10 DAT with dicamba in 2020 displayed the mentioned symptoms (Figure 6), but with the difference that small plants showed severe auxin symptoms at the same rate as larger ones.

The Arkansas Agriculture Department provided results of dicamba detection from a Palmer amaranth sample collected on July 26, 2019, in Phillips County, AR (34.56 N, 90.81 W), that was allegedly damaged by dicamba drift. Analytical results equivalent to 23.68 µg g^{-1} of dicamba were recovered on the sample (file number 236-2019), collected 62 d after the state-determined cutoff date for a labeled dicamba application, which was May 25, 2019 (Steed 2019). This research did not perform herbicide detection tests longer than 1 mo after application; however, based on these data, the possibility of recovering similar concentrations of dicamba occurred only for treatment that received a field-labeled rate of the herbicide (1X) and only on the day of application. A possible hypothesis is that this field likely received an illegal application (after the cutoff) of a 1X or higher rate of dicamba.

Field symptomology results indicate that growers are unlikely to notice plant symptoms originating from off-target movement of a single exposure of auxin herbicides of rates lower than 0.1X and would be unlikely to report to regulatory authorities. Severe auxin symptoms on Palmer amaranth required a full rate to develop. Results of this experiment imply that when inquiries related to

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

Figure 6. Small, medium, and large Palmer amaranth (which corresponded to 8 to 15 cm, 20 to 30 cm, and 35 to 50 cm in height at application) at 10 d after treatment with dicamba at 560 g ae ha^{-1} (A–C, respectively), 56 g ae ha^{-1} (D–F, respectively), and 5.6 g ae ha^{-1} (G–I, respectively) in 2020.

plant damage by herbicide are made, particularly for dicamba or 2,4-D, it would be possible to detect herbicide residue on plant tissue primarily if a direct application (1X rate) occurred, even up to a month after application. It is important to note that a single exposure of Palmer amaranth plants to simulated particle drift rates (0.1X rate or lower) of 2,4-D or dicamba often resulted in no detection of herbicide (Supplementary Tables S1 and S2) and only slight petiole curvature and symptomology; therefore it could be challenging to determine when the exposure occurred.

Dicamba and 2,4-D Residues in Xtend® and Enlist® Soybean

The concentration of dicamba and 2,4-D residue in soybean tissue analyzed after treatment with these herbicides varied by year, soybean trait, herbicide rate applied, and time after application. Therefore herbicide recovery data are displayed separately by herbicide treatment, year, soybean trait, and rate applied. The concentrations of 2,4-D and dicamba detected on soybean on the day of application (0 DAT) varied substantially by herbicide rate applied (Figures 7 to 10). For instance, results varied substantially for 1X treatment (560 g ae ha^{-1} of dicamba or 1,065 g ae ha^{-1} of 2,4-D); the detection in 2020 was from 18 to 157 µg g^{-1} of dicamba and from 27 to 120 µg g^{-1} of 2,4-D, while a variation between 8 and 16 µg g^{-1} of dicamba and 14 and 150 µg g^{-1} of 2,4-D occurred in 2021 (Figures 7 to 10; Supplementary Tables S3 to S6). The variation in herbicide detection on the day of application is attributed to differing levels of spray interception among plants within the row and the fact that sampling occurred randomly within each plot row treated.

Like the Palmer amaranth study, the recovered herbicide concentration declined rapidly after soybean plants were treated with dicamba and 2,4-D, regardless of year, soybean trait, or herbicide rate applied. Considering the total number of samples analyzed for dicamba (640 samples), no residue could be detected

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

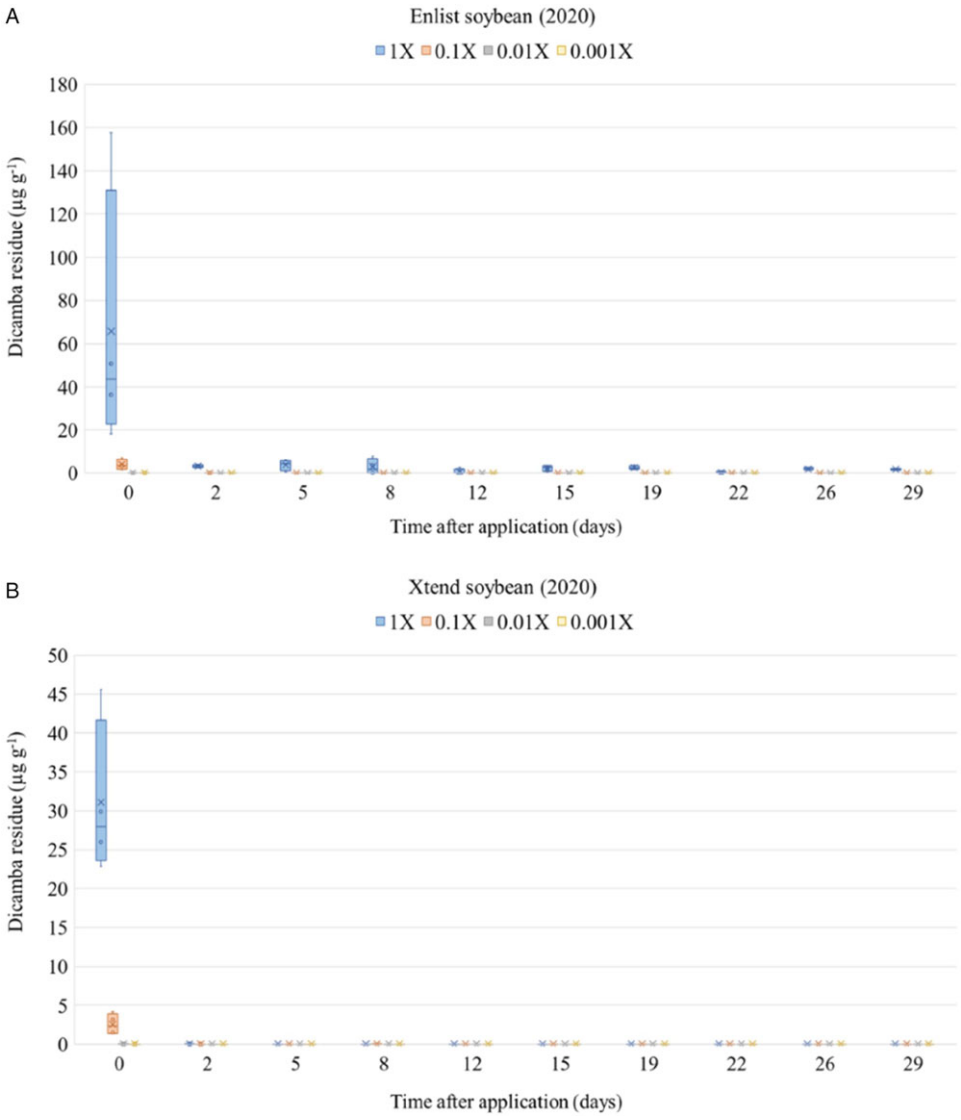

Figure 7. Distribution of dicamba residue (µg g^{-1}) in Enlist® (A) and Xtend® (B) soybean detected over time after application with dicamba at 1X, 0.1X, 0.01X, and 0.001X rates (blue, orange, gray, and yellow boxes, respectively) in 2020, with 1X being 560 g ae ha^{-1}. Dicamba residue in Enlist® soybean in 2020 regressed as a function of time after application using the equations $Y_{1X} = \exp(3.99 - 0.66X)$ (generalized $R^2 = 0.83$) and $Y_{0.1X} = \exp(1.39 - 3.31X)$ (generalized $R^2 = 0.62$). Dicamba residue in Xtend® soybean in 2020 regressed as a function of time after application using the equations $Y_{1X} = \exp(3.35 - 3.18X)$ (generalized $R^2 = 0.81$) and $Y_{0.1X} = \exp(0.95 - 3.01X)$ (generalized $R^2 = 0.63$). Relationships were not significant for Enlist® or Xtend® soybean treated with dicamba at ≤0.01X.

in 549 samples, of which 158 and 153 were initially treated with 0.001X and 0.01X rates of dicamba over both years. Laboratory results of 2,4-D analysis resulted in no residue detected in 449 samples; 143 and 136 samples were treated with a 0.001X and 0.01X rate of 2,4-D, respectively (Supplementary Tables S3 to S6). Warm conditions (air temperature between 20 C and 30 C) and rainfall probably resulted in accelerated metabolism of both herbicides, reducing the ability to detect the active form of dicamba or 2,4-D in the soybean tissue. A 22-mm rainfall occurred 2 DAT in 2020, prior to the second sampling, while two rain events that accumulated 26 mm occurred before the second collection in 2021 (4 DAT) (Figure 2 B and C). With results averaged over soybean traits, herbicide detected between 0 DAT and at the second collection (2 DAT for 2020 and 4 DAT for 2021) decayed more similarly in both years than in the experiment with Palmer amaranth; for instance, on average, 2,4-D reduced 70 µg g^{-1} in 2020 and 2021, and dicamba reduced, on average, 33 µg g^{-1} and 12 µg g^{-1} between the first and second samplings after treatment with a 1X rate in 2020 and 2021, respectively (Figures 7 to 10). Additionally, the Enlist® soybean was able to degrade 2,4-D, whereas the Xtend® soybean is resistant to dicamba, and therefore treatments with these herbicides do not cause a prolonged impact on soybean with these traits (Nandula 2019).

Generalized regression results analyzed separately for dicamba and for 2,4-D treatment as a function of time after application differed by year, soybean trait, and herbicide rate. Regression curves for Enlist® soybean treated with dicamba had generalized R^2 values ranging from 0.62 to 0.83 in 2020 and from 0.24 to 0.41 in 2021 (Figures 7 and 8). Overall, inverse prediction results show that dicamba-treated Enlist® soybean at 560 g ae ha^{-1} and 56 g ae ha^{-1} (simulating a 1X and a 0.1X treatment, respectively) resulted in detections up to 31 and 16 DAT, respectively (data not

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

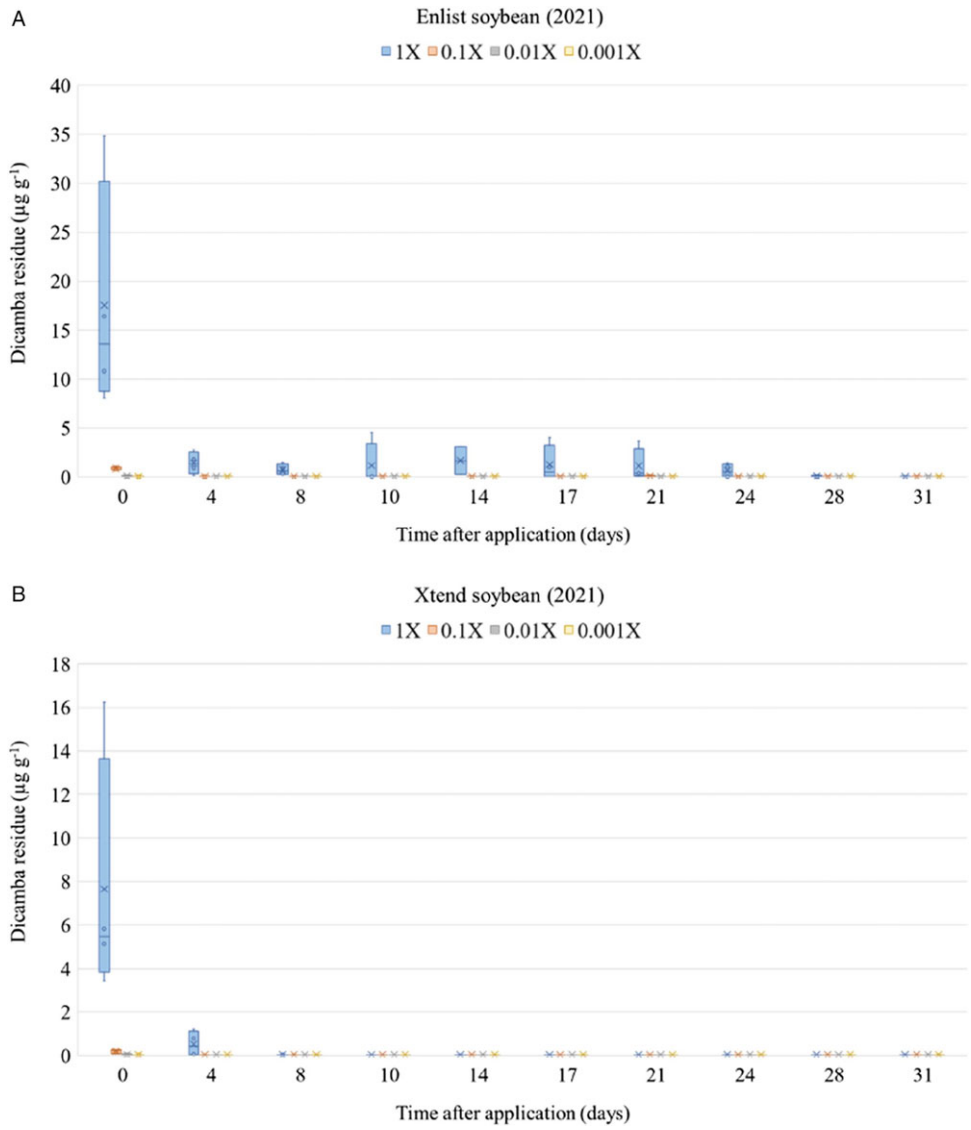

Figure 8. Distribution of dicamba residue (µg g^{-1}) in Enlist® (A) and Xtend® (B) soybean detected over time after application with dicamba at 1X, 0.1X, 0.01X, and 0.001X rates (blue, orange, gray, and yellow boxes, respectively) in 2021, with 1X being 560 g ae ha^{-1}. Dicamba residue in Enlist® soybean in 2021 regressed as a function of time after application using the equations $Y_{1X} = \exp(3.67 - 0.19X)$ (generalized $R^2 = 0.41$) and $Y_{0.1X} = \exp(-0.70 - 0.16X)$ (generalized $R^2 = 0.24$). Dicamba residue in Xtend® soybean in 2021 regressed as a function of time after application using the equation $Y_{1X} = \exp(2.43 - 0.82X)$ (generalized $R^2 = 0.71$). Relationships were not significant for Enlist® or Xtend® soybean treated with dicamba at ≤0.01X and at ≤0.1X, respectively.

shown). Regression curves for Xtend® soybean treated with dicamba had generalized R^2 values ranging from 0.63 to 0.81 in 2020; meanwhile, for the 2021 result, only the 1X relationship was significant, with a generalized R^2 equal to 0.71 (Figures 7 and 8). Overall, inverse prediction results show that dicamba-treated Xtend® soybean at 560 g ae ha^{-1} and 56 g ae ha^{-1} (simulating a 1X and a 0.1X treatment, respectively) resulted in detections up to 6 and 1 DAT, respectively (data not shown). Enlist® and Xtend® soybean did not result in appropriate regression curves after dicamba ≤ 5.6 g ae ha^{-1} in 2020 or ≤ 5.6 g ae ha^{-1} and 56 g ae ha^{-1}, respectively, in 2021 (generalized $R^2 < 0.2$; data not shown). Regression curves for Enlist® soybean treated with 2,4-D had generalized R^2 values ranging from 0.58 to 0.77 in 2020 and from 0.76 to 0.77 in 2021 (Figures 9 and 10). Inverse predictions showed that 2,4-D-treated Enlist® soybean at 1,065 g ae ha^{-1} and 106.5 g ae ha^{-1} (simulating a 1X and a 0.1X treatment, respectively) resulted in detections up to 15 and 9 DAT, respectively (data not shown). Regression curves for Xtend® soybean treated with 2,4-D had generalized R^2 values ranging from 0.60 to 0.80 in 2020 and from 0.74 to 0.87 in 2021 (Figures 9 and 10). Inverse predictions showed that 2,4-D-treated Xtend® soybean at 1,065 g ae ha^{-1} and 106.5 g ae ha^{-1} (simulating a 1X and a 0.1X treatment, respectively) resulted in detections up to 30 and 16 DAT, respectively (data not shown). Enlist® and Xtend® soybean did not result in appropriate regression curves at 2,4-D rates ≤ 10.65 g ae ha^{-1} in either year (generalized $R^2 < 0.2$; data not shown). Prior research conducted using growth chambers recovered only 0.02 µg g^{-1} of dicamba at 7 d after treating conventional soybean plants with the herbicide at 0.5 g ae ha^{-1}, and no detection occurred by 28 DAT; detection was consistent up to 35 DAT only for plants treated with 50 g ae ha^{-1} (Sirons et al. 1982). The same researchers reported that 2,4-D recovered from conventional soybean showed that only

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

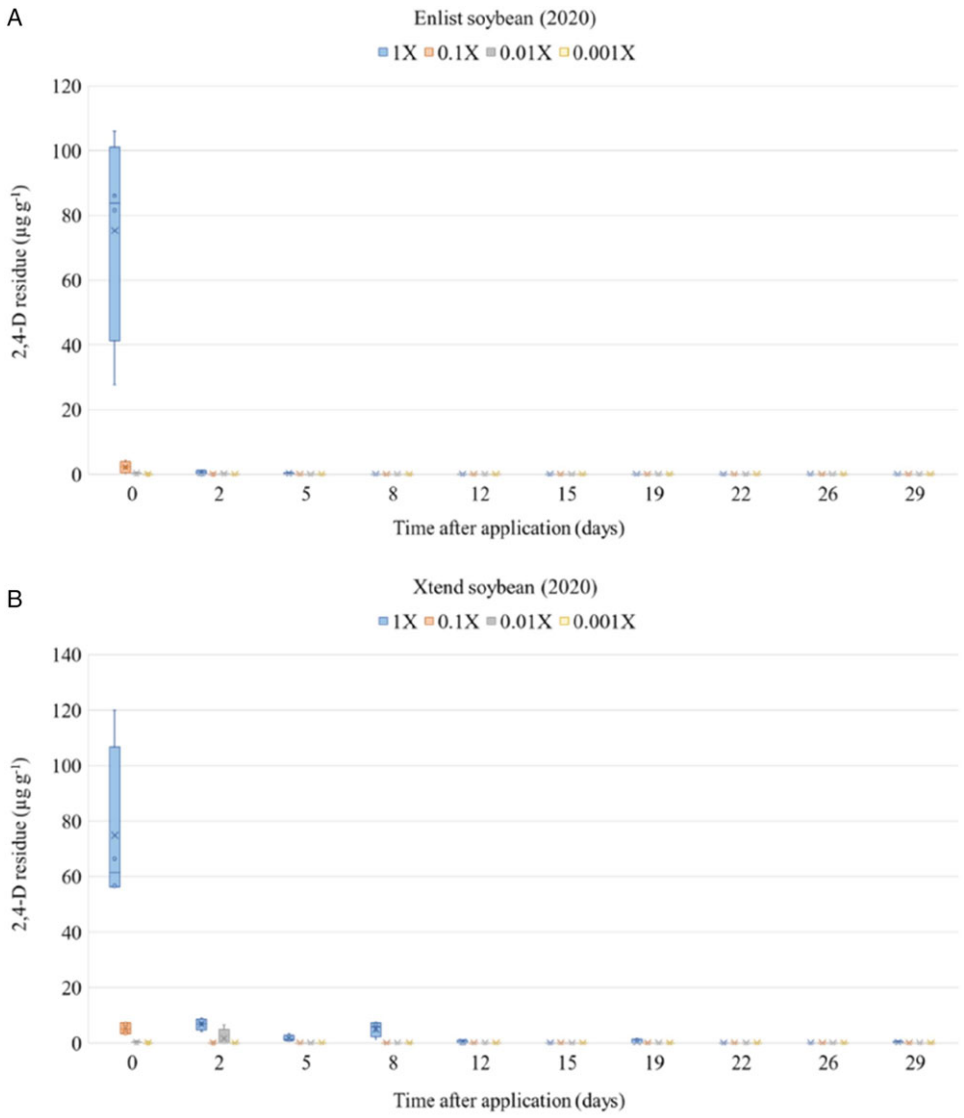

Figure 9. Distribution of 2,4-D residue (µg g^{-1}) in Enlist® (A) and Xtend® (B) soybean detected over time after application with 2,4-D at 1X, 0.1X, 0.01X, and 0.001X rates (blue, orange, gray, and yellow boxes, respectively) in 2020, with 1X being 1,065 g ae ha^{-1}. 2,4-D residue in Enlist® soybean in 2020 regressed as a function of time after application using the equations $Y_{1X} = \exp(3.89 - 0.51X)$ (generalized $R^2 = 0.77$) and $Y_{0.1X} = \exp(1.19 - 0.74X)$ (generalized $R^2 = 0.58$). 2,4-D residue in Xtend® soybean in 2020 regressed as a function of time after application using the equations $Y_{1X} = \exp(5.72 - 0.35X)$ (generalized $R^2 = 0.60$) and $Y_{0.1X} = \exp(1.25 - 0.99X)$ (generalized $R^2 = 0.80$). Relationships were not significant for Enlist® or Xtend® soybean treated with 2,4-D at ≤0.01X.

trace amounts were found at 7 DAT following application with 5 g ae ha^{-1}.

During this research, photographs were taken to document auxin injury symptomology on Enlist® and Xtend® soybean treated with dicamba or 2,4-D. Visible injury occurred as early as the second sampling (at 2 or 4 DAT, depending on the year). As expected, visible symptoms were stem twisting, epinasty, and apical meristem malformation, often resulting in lodging for Enlist® soybean treated with dicamba at 1X and 0.1X rates; Xtend® soybean was not impacted, nor was Enlist® soybean treated with rates lower than 0.1X (Figure 11). Similar symptoms were observed on Xtend® soybean treated with 2,4-D at 1X and 0.1X rates, whereas no damage to Enlist® soybean or Xtend® soybean treated with rates lower than 0.1X was observed (Figure 12). The concentrations of both herbicides declined rapidly in soybean and Palmer amaranth over time, whereas the extent of injury to soybean and Palmer amaranth, especially at less than a fully labeled rate, is slow to manifest as visible symptoms. This phenomenon points to the difficulty that pesticide agency regulators have in positively identifying an illegally treated field with either 2,4-D or dicamba and the need to match symptomology or extent of damage with the concentration of the herbicide detected in plants.

The general appearance of soybean plants treated with a 0.01X or 0.001X rate of either herbicide indicates that it is unlikely that growers will notice injury from a single low-dose exposure and, therefore, that they would be unlikely to make an official complaint with pesticide regulatory authorities. Laboratory analysis to detect auxin herbicides on soybean tissue could be used to distinguish between a single exposure to a direct application and other forms of exposure, particularly if sampling was made within a couple weeks of the incident. Higher-than-labeled treatment or multiple exposures to auxin herbicides could result in detection several weeks after application. Hence documenting plant symptoms and the chemical analysis of auxin residues in Palmer amaranth

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

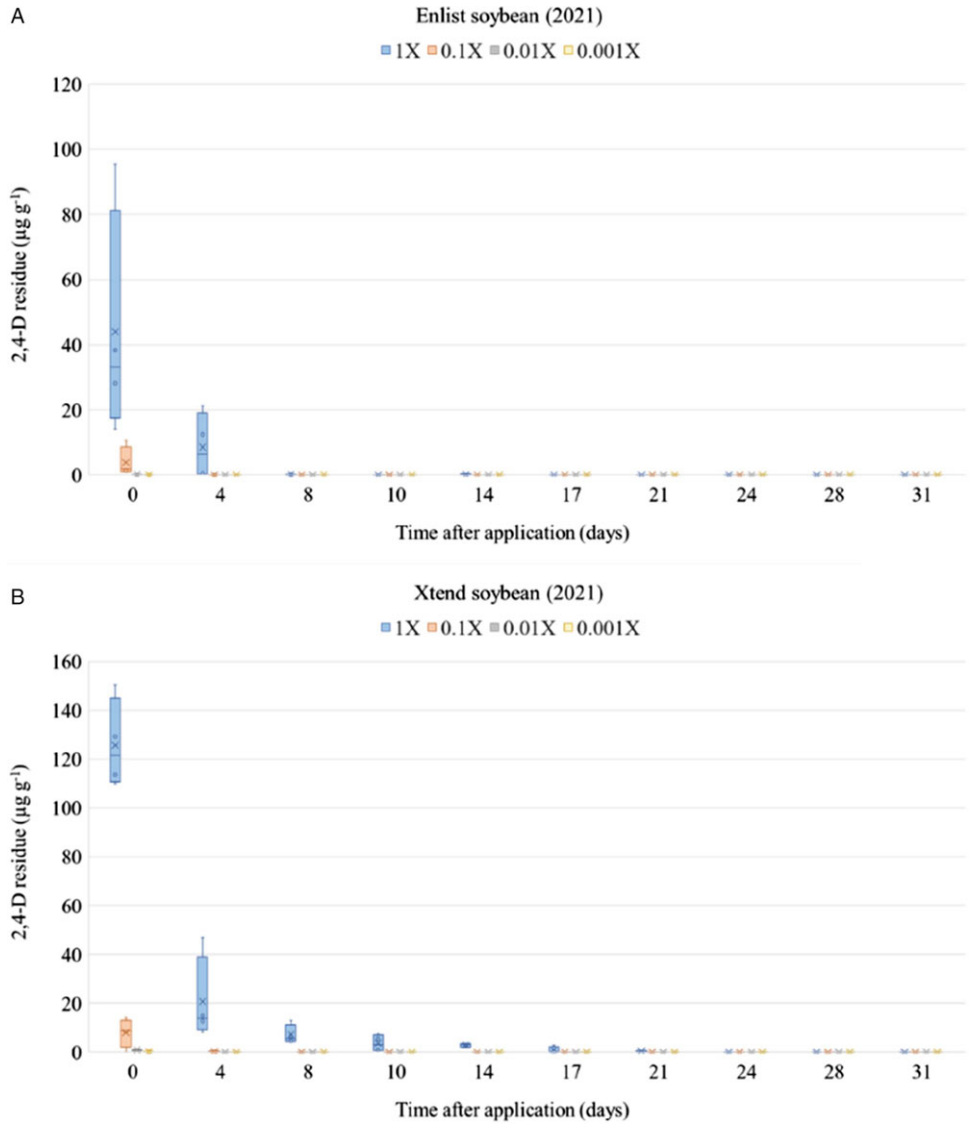

Figure 10. Distribution of 2,4-D residue (µg g^{-1}) in Enlist® (A) and Xtend® (B) soybean detected over time after application with 2,4-D at 1X, 0.1X, 0.01X, and 0.001X rates (blue, orange, gray, and yellow boxes, respectively) in 2021, with 1X being 1,065 g ae ha^{-1}. 2,4-D residue in Enlist® soybean in 2021 regressed as a function of time after application using the equations $Y_{1X} = \exp(5.41 - 0.69X)$ (generalized $R^2 = 0.77$) and $Y_{0.1X} = \exp(1.14 - 1.20X)$ (generalized $R^2 = 0.76$). 2,4-D residue in Xtend® soybean in 2021 regressed as a function of time after application using the equations $Y_{1X} = \exp(5.94 - 0.38X)$ (generalized $R^2 = 0.87$) and $Y_{0.1X} = \exp(1.89 - 0.44X)$ (generalized $R^2 = 0.74$). Relationships were not significant for Enlist® or Xtend® soybean treated with 2,4-D at ≤0.01X.

or soybean could help to determine when applications occurred and what rate of dicamba or 2,4-D was applied. It is important to note that residue tolerance guidelines of dicamba and 2,4-D on food or fodder have been established following international standards from the European Food Safety Authority; for instance, 60 µg g^{-1} of dicamba and 2 µg g^{-1} of 2,4-D are tolerated on soybean forage, while seed concentrations were 10 µg g^{-1} and 0.02 µg g^{-1}, respectively (U.S. Environmental Protection Agency 2022). Furthermore, pesticide residue above the maximum residue limit leads to economic losses, as the crop cannot be marketed, regardless of the source of the contamination.

Practical Implications

Pesticide agency officials collect plant tissue samples and document symptomology to investigate potential sources of off-target movement of pesticides, particularly dicamba and 2,4-D. The research reported here shows that the presence of auxin-like symptoms on Palmer amaranth and soybean plants combined with the detection of residues of 2,4-D and dicamba suggests an earlier direct application rather than off-target movement. Time of collection following exposure influences the ability to detect herbicides. Generally, treatments of dicamba ≤ 5.6 g ae ha^{-1} and 2,4-D ≤ 10.65 g ae ha^{-1} resulted in slight symptoms in Palmer amaranth that could be unnoticeable 5 d after application. Environmental conditions, particularly temperature, sunlight availability, and rainfall, can impact herbicide persistence. Overall, dicamba and 2,4-D were better detected later following treatment in Palmer amaranth than in both soybean technologies tested, indicating that investigative plant collections should include weeds in the field. More research is needed to evaluate how multiple exposures to dicamba and 2,4-D, including volatilization, affect residue detection.

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press

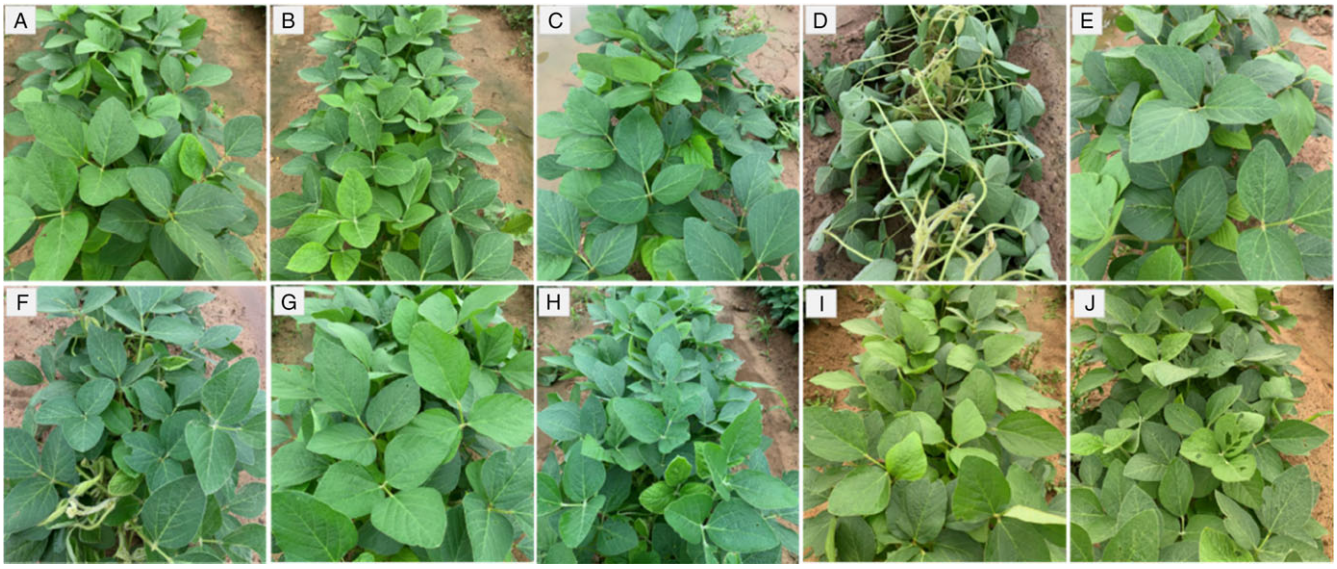

Figure 11. Nontreated Xtend® (A) and Enlist® (B) plots and treatments with dicamba at 560, 56, 5.6, and 0.56 g ae ha^{-1} on Xtend® (C, E, G, and I, respectively) and on Enlist® soybean (D, F, H, and J, respectively) 3 d after treatment in 2021.

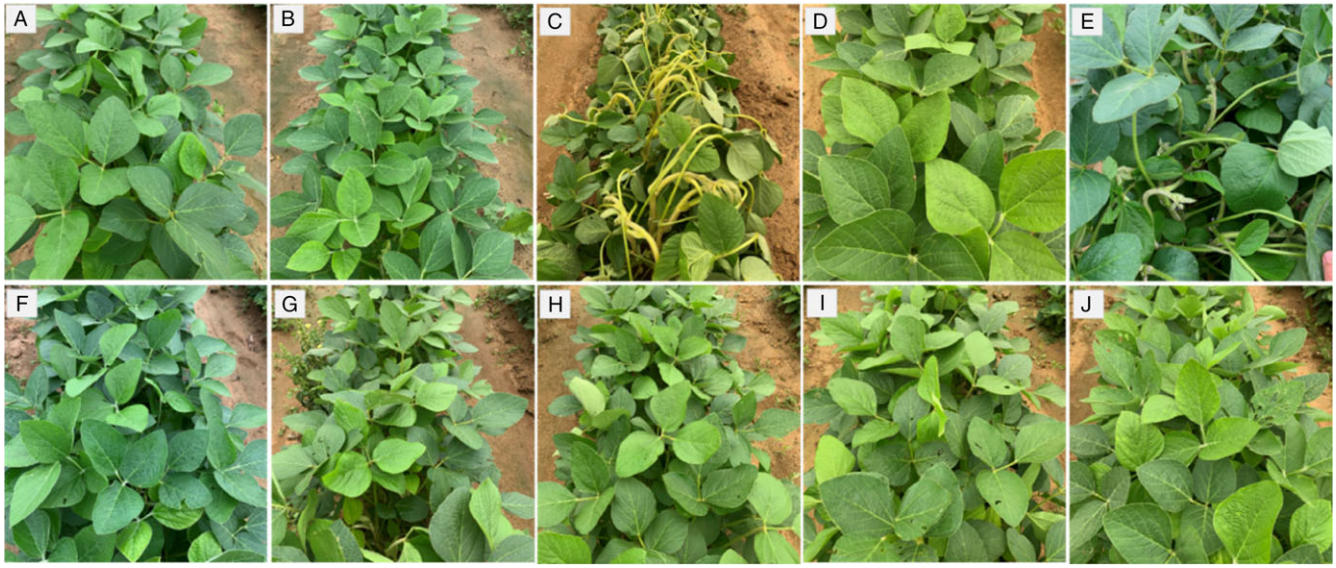

Figure 12. Nontreated Xtend® (A) and Enlist® (B) plots and treatments with 2,4-D at 1,065, 106.5, 10.65, and 1.065 g ae ha^{-1} on Xtend® (C, E, G, and I, respectively) and on Enlist® soybean (D, F, H, and J, respectively) 3 d after treatment in 2021.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2023.60

Acknowledgments. The Arkansas Agriculture Department provided financial support for this research. The authors gratefully acknowledge the Arkansas Agriculture Department, Pesticide Division personnel responsible for the laboratory analysis performed with plant samples collected in the field. The authors declare no conflicts of interest.

References

Akesson NB, Yates WE (1964) Problems relating to application of agricultural chemicals and resulting drift residues. Annu Rev Entomol 9:285–318

Andersen SM, Clay SA, Wrage LJ, Matthees D (2004). Soybean foliage residues of dicamba and 2,4-D and correlation to application rates and yield. Agron J 96:750–760

Anonymous (2022a) Engenia® herbicide label. BASF Corporation Publication Number 7969-472. Research Triangle Park, NC: BASF. 21 p

Anonymous (2022b) Enlist One® Herbicide with Colex D Technology herbicide label. Corteva Agriscience Publication Number 62719-695. Indianapolis, IN: Corteva Agriscience. 7 p

Anonymous (2022c) Enlist Duo® Herbicide with Colex D Technology herbicide label. Corteva Agriscience Publication Number 62719-649. Indianapolis, IN: Corteva Agriscience. 8 p

Anonymous (2022d) Tavium® Plus VaporGrip® Technology herbicide label. Syngenta Crop Protection Publication Number 100-1623. Greensboro, NC: Syngenta Crop Protection. 28 p

Anonymous (2022e) XtendiMax® Herbicide with VaporGrip® Technology herbicide label. Bayer Crop Science Publication Number 264-1210. St. Louis, MO: Bayer Crop Science. 13 p

Arneson N, Werle R (2020) Enlist E3 soybean system in 2020: what we think applicators should know. https://ipcm.wisc.edu/blog/2020/04/enlist-e3-system-in-2020-what-we-think-applicators-should-know/. Accessed: December 1, 2022

Auch DE, Arnold WE (1978) Dicamba use and injury on soybeans (*Glycine max*) in South Dakota. Weed Sci 26:471–475

Behrens R, Lueschen WE (1979) Dicamba volatility. Weed Sci 27:486–493

Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, LaVallee BJ, Herman PL, Clemente TE, Weeks DP (2007) Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies. Science 316:1185–1188

Bish M, Oseland E, Bradley K (2021) Off-target pesticide movement: a review of our current understanding of drift due to inversions and secondary movement. Weed Technol 35:345–356

Bradley KW (2017) How to proceed in 2018: a university perspective. Page 246 *in* Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis, MO: North Central Weed Science Society

Bradley K (2018) Dicamba injury update: different year, same questions. https://ipm.missouri.edu/cropPest/2018/7/July-15-Dicamba-injury-update-different-year-same-questions/. Accessed: October 29, 2022

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. Weed Technol 29:758–770

Chamkasem N, Morris C (2016) Determination of 2,4-dichlorophenoxyacetic acid in soybean and corn by liquid chromatography/tandem mass spectrometry. J Regul Sci 4:9–18

Grossmann K (2010) Auxin herbicides: current status of mechanism and mode of action. Pest Manag Sci 66:113–120

Hartzler B, Jha P (2020) What went wrong in Iowa? https://crops.extension.iastate.edu/blog/bob-hartzler-prashant-jha/dicamba-2020-what-went-wrong-iowa. Accessed: August 25, 2022

Heap I (2023) International survey of herbicide resistance. http://www.weedscience.org/. Accessed: January 5, 2023

Jones GT, Norsworthy JK, Barber T, Gbur E, Kruger GR (2019) Off-target movement of DGA and BAPMA dicamba to sensitive soybean. Weed Technol 33:51–65

Maybank J, Yoshida K, Grover R (1978) Spray drift from agricultural pesticide applications. J Air Pollut Control Assoc 28:1009–1014

Meyer C, Norsworthy J, Young BG, Steckel L, Bradley K, Johnson W, Loux M, Davis VM, Kruger G, Bararpour MT, Ikley J, Butts T (2015) Herbicide program approaches for managing glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus* and *Amaranthus rudis*) in future soybean-trait technologies. Weed Technol 29:716–729

Meyer CJ, Peter F, Norsworthy JK, Beffa R (2020) Uptake, translocation, and metabolism of glyphosate, glufosinate, and dicamba mixtures in *Echinochloa crus-galli* and *Amaranthus palmeri*. Pest Manag Sci 76:3078–3087

Mueller TC, Wright DR, Remund KM (2013) Effect of formulation and application time of day on detecting dicamba in the air under field conditions. Weed Sci 61:586–593

Nagelkerke NJD (1991) A note on a general definition of the coefficient of determination. Biometrika 78:691–692

Nandula VK (2019) Herbicide resistance traits in maize and soybean: current status and future outlook. Plants 8:337

Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW (2016) 2,4-D past, present, and future: a review. Weed Technol 30:303–345

SAS Institute Inc. (2022) Discovering JMP 17. Cary, NC: SAS Institute Inc. 240 p

Schmidt GM (2019) Corteva Agriscience™ announces Enlist E3™ soybean and Qrome® corn commercial launches. https://www.corteva.us/press-releases/corteva-agriscience-announces-enlist-e3-soybean-and-qrome-corn-commercial-launches.html. Accessed: October 1, 2023

Shaner DL, ed. (2014) Herbicide Handbook. 10th ed. Lawrence, KS: Weed Science Society of America. 513 p

Sirons GJ, Anderson GW, Frank R, Ripley BD (1982) Persistence of hormone-type herbicide residue in tissue of susceptible crop plants. Weed Sci 30: 572–578

Soltani N, Oliveira MC, Alves GS, Werle R, Norsworthy JK, Sprague CL, Young BG, Reynolds DB, Brown A, Sikkema PH (2020) Off-target movement assessment of dicamba in North America. Weed Technol 34:318–330

Sosnoskie LM, Culpepper AS, Braxton LB, Richburg JS (2015) Evaluating the volatility of three formulations of 2,4-D when applied in the field. Weed Technol 29:177–184

Spaunhorst DJ, Johnson WG (2017) Variable tolerance among Palmer amaranth (*Amaranthus palmeri*) biotypes to glyphosate, 2,4-D amine, and premix formulation of glyphosate plus 2,4-D choline (Enlist Duo®) herbicide. Weed Sci 65:787–797

Steckel L (2018) Dicamba in Tennessee: year 3. https://news.utcrops.com/2018/07/dicamba-in-tennessee-year-3/. Accessed: August 23, 2022

Steckel L (2019) Houston, we have a problem. https://soybeansouth.com/departments/feature/houston-we-have-a-problem/. Accessed: August 24, 2022

Steckel L, Bond J, Ducar J, York A, Scott B, Dotray P, Barber T, Bradley K (2017) The good the bad and the ugly: Dicamba observations of southern weed extension scientists. Pages 98–99 *in* Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis, MO: North Central Weed Science Society

Steed S (2019) Panel backs May 25 cutoff for dicamba. https://www.arkansasonline.com/news/2019/feb/27/news-in-brief-20190227/?business. Accessed: December 8, 2022

Timmons F (1970) A history of weed control in the United States and Canada. Weed Sci 18:294–307

Troyer J (2001) In the beginning: the multiple discovery of the first hormone herbicides. Weed Sci 49:290–297

U.S. Environmental Protection Agency (2022) 40 CFR part 180: tolerances and exemptions from tolerances for pesticide chemicals in food. https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-180. Accessed: January 12, 2023

Van Wychen L (2022) 2022 survey of the most common and troublesome weeds in broadleaf crops, fruits and vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. https://wssa.net/wp-content/uploads/2022-Weed-Survey-Broadleaf-crops.xlsx. Accessed: September 29, 2023

Wechsler S (2018) Trends in the adoption of genetically engineered corn, cotton, and soybeans. https://www.ers.usda.gov/amber-waves/2018/december/trends-in-the-adoption-of-genetically-engineered-corn-cotton-and-soybeans/. Accessed: September 10, 2022

Werle R, Mobli A, Striegel S, Arneson N, DeWerff R, Brown A, Oliveira M (2022) Large-scale evaluation of 2,4-D choline off-target movement and injury in 2,4-D-susceptible soybean. Weed Technol 36:8–14

Werle R, Oliveira MC, Jhala AJ, Proctor CA, Rees J, Klein R (2018) Survey of Nebraska farmers' adoption of dicamba-resistant soybean technology and dicamba off-target movement. Weed Technol 32:754–761

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P, Zhuang M, Arnold NL, Lin G, Yau K, Russell SM, Cicchillo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z (2010) Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proc Natl Acad Sci U S A 107:20240–20245

Zimdahl RL (2013) Fundamentals of Weed Science. 4th ed. Waltham, MA: Academic Press. 648 p

https://doi.org/10.1017/wet.2023.60 Published online by Cambridge University Press