Weed Technology

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Response of dicamba-resistant soybean cultivars to postemergence dicamba dose exposure

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Research Article

Cite this article: Larini WF, Albrecht AJP, Neuberger DC, Fischer GH, Albrecht LP, Barroso AM (2025) Response of dicamba-resistant soybean cultivars to postemergence dicamba dose exposure. Weed Technol. **39**(e95), 1–11. doi: 10.1017/wet.2025.10046

Received: 4 June 2025
Revised: 30 August 2025
Accepted: 4 September 2025

Associate Editor:
Prashant Jha, Louisiana State University

Nomenclature:
Dicamba; soybean; *Glycine max* (L.) Merr.

Keywords:
Auxinic herbicides; *dmo* gene; herbicide exposure; soybean; *Glycine max* (L.) Meer; xtendicam

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Abstract

Dicamba-resistant (DR) soybean cultivars are essential elements in managing broadleaf weeds in modern production systems. However, limited information is available regarding yield reductions associated with dicamba rates that were previously registered for postemergence weed control and off-label dicamba rates in these cultivars. This study aimed to characterize and quantify the effects of postemergence dicamba applications on two DR soybean cultivars. Field trials were conducted in 2022 and 2023, with dicamba applied at 0 to 1,440 g ae ha^{-1} during the V5 to V6 stages. Visible injury increased with dicamba rate, reaching 18% (Cultivar A) to 20% (Cultivar B) at 1,440 g ae ha^{-1} at 3 d after treatment, but symptoms declined to <10% by 4 wk after treatment (WAT). Chlorophyll fluorescence was not significantly affected at 2 and 4 WAT. Height reduction at 4 WAT occurred only at the highest dicamba rate (1,440 g ae ha^{-1}), but differences disappeared by maturity. Dry biomass reduction was also dose-dependent, reaching 16% for Cultivar A and 10% for Cultivar B at the highest rate. Pod reduction in DR soybean was minor (<3.5%) and not significant. Applications of dicamba from 288 to 864 g ae ha^{-1} resulted in minimal yield reductions (<5%) and no significant biomass reduction. At a dicamba dose of 1,152 g ae ha^{-1}, yield reductions reached 7% and 9% for Cultivars A and B, respectively, while the highest rate (1,440 g ae ha^{-1}) resulted in yield reductions of 12% (Cultivar A) and 14% (Cultivar B). Despite over-the-top application restrictions, these results confirm that DR soybean cultivars tolerate rates (≤720 g ae ha^{-1}) of dicamba that were previously registered for postemergence weed control with minimal (<5%) yield reduction and recover rapidly from transient injury. However, applications above these rates can reduce yield by up to 14%, highlighting the importance of adhering to recommended dicamba use guidelines.

Introduction

Dicamba-resistant (DR) soybean, developed through the insertion of the dicamba monooxygenase (*dmo*) gene that enables dicamba metabolism and provides herbicide tolerance (ISAAA 2024), has been commercially available to growers in the United States since 2016 (Wechsler 2018) and in Brazil since 2022 (CTNBio 2017), and this genetically engineered trait has been widely adopted by soybean producers in both countries. By 2018, DR soybean cultivars accounted for approximately 43% of U.S. soybean acreage, with an estimated 22.3 million hectares planted annually (US EPA 2021; USDA-ERS 2022; Wechsler 2018).

Market research shows that approximately 60% of DR soybean fields received at least one dicamba application, with up to 8.9 million ha planted primarily as a safeguard against off-target exposure rather than for direct weed control (US EPA 2021). Adoption from 2016 to 2018 followed a trajectory similar to that of glyphosate-resistant varieties in the late 1990s, particularly in areas with widespread glyphosate-resistant weeds. The global dicamba herbicide market was valued at US$559.5 million in 2023 and is projected to grow at an annual rate of 7.9% through 2030, primarily driven by its efficacy in controlling herbicide-resistant broadleaf weeds and the increasing adoption of DR crops, such as soybean and cotton (Grand View Research 2025).

Dicamba (3,6-dichloro-2-methoxybenzoic acid) has been used as herbicide since 1967 in both agricultural and nonagricultural fields to target annual, biennial, and perennial broadleaf weeds in a range of food and feed crops (Busi et al. 2018; US EPA 2024). In soybean production, dicamba plays a critical role in the management of herbicide-resistant broadleaf species and has been widely investigated for both its weed control performance and the potential for its off-target movement (Carbonari et al. 2022; Egan and Mortensen 2012; Mueller and Steckel 2019; Riter et al. 2021; Sall et al. 2020). The growing role of dicamba applied either before or after the crop emerges, but primarily to control emerged weeds, is reflected in its significant increase in use in both Brazil and the United States. In the United States, dicamba usage grew by 300% from

2013 to 2017 (USGS 2024), while in Brazil, sales of dicamba (acid equivalent) rose by more than 1,200%, from 11,600 kg in 2018 to 155,900 kg in 2022 (IBAMA 2023). These increases were driven by the widespread adoption of DR soybean cultivars and the growing resistance of weeds to 2,4-D in both countries. Yet, recent regulatory revocations no longer permit over-the-top (OTT) dicamba applications to soybean under current label recommendations and restrictions. Even so, generating exploratory data under controlled research conditions remains necessary if we are to better understand DR soybean responses and to inform future regulatory discussions.

Injury symptoms associated with dicamba exposure typically include crinkling and cupping of young leaves, epinasty, stem twisting, leaf droop, reduced plant height, apical meristem necrosis, abnormal pod development, and ultimately, decreased grain yield (Andersen et al. 2004; Canella Vieira et al. 2022; Grossmann, 2010; Kniss 2018; Weidenhamer et al. 1989). A meta-analysis shows that soybean plants are highly variable in their responses to dicamba exposure (Kniss 2018). The visible injury symptoms, especially during vegetative stages, are not predictive of final yield reduction (Egan et al. 2014; Garcia et al. 2025). The U.S. Environmental Protection Agency (U.S. EPA) has assessed the relationship between visible injury symptoms such as leaf crinkling, cupping, and epinasty; plant height; and yield across multiple studies and determined that a 10% visible injury threshold is a sensitive and protective endpoint, typically associated with a reduction in growth or yield of less than 5% in most cases (US EPA 2020). Therefore, this study adopted the 5% and 10% visible injury and yield reductions levels as recommended by the U.S. EPA and previous studies (Kniss 2018; Price et al. 2017), to characterize the severity of dicamba-induced injury and assess the sensitivity of DR soybean cultivars under increasing dicamba doses.

Nonresistant soybean plants are highly sensitive to dicamba, exhibiting symptoms even at ultralow doses such 0.03 g ae ha^{-1}, the lowest non-zero dose reported in the literature; consequently, significant injury might be expected at slightly lower rates (Foster and Griffin 2018; Kniss, 2018; Riter et al. 2021; Solomon and Bradley 2014). In contrast, DR cultivars that express the *dmo* gene exhibit a much higher tolerance, but transient injury can still occur shortly after a herbicide is applied. While the genetic resistance of soybean to dicamba prevents major injury, postemergence application of dicamba at 720 g ae ha^{-1} may cause slight reductions in net CO_2 assimilation without compromising carboxylation efficiency. These mild physiological effects likely result from early herbicide perception and hormonal signaling. The *dmo* trait effectively metabolizes dicamba into 3,6-dichlorosalicylic acid (3,6-DCSA), with metabolite levels exceeding those of dicamba within 3 d after application and internal dicamba concentrations dropping below 0.05 g ha^{-1} within 24 h (Pereira 2023). Moreover, dicamba applied at 600 g ai ha^{-1} reduced the yield of DR soybean by 5.6%, a figure that was not statistically different from that of the nontreated plots, resulting in no negative effect on soybean yield (Underwood et al. 2016). These findings highlight the effectiveness of the *dmo* gene in mitigating dicamba injury, while underscoring the need for further research on its impact on yield components.

Although DR soybean is a key tool in weed management, much of the existing research has focused on off-target drift or sublethal exposures. With newer DR cultivars exhibiting higher yield potential, shorter growth cycles, and increased nutrient demands, understanding how these traits influence their tolerance to dicamba is crucial, even considering current restrictions on OTT dicamba applications. Despite the potential variability in responses among DR cultivars, limited information exists on the severity of symptoms at different dicamba application rates, leading to uncertainty about how much dicamba DR soybean can tolerate without compromising performance. Therefore, the results presented here should be interpreted as exploratory, and intended to advance scientific understanding of DR soybean responses to dicamba. They are not an endorsement of nonlabeled use, but rather we expect the results to contribute evidence that may support future discussions on dicamba regulation and management in soybean production systems. This study aims to characterize the effects of postemergence dicamba doses on DR soybean cultivars.

Material and Methods

Experimental Site and Design

Field trials assessing DR soybean response to dicamba dose exposure were conducted during the 2022 and 2023 growing seasons in no-till agricultural fields previously cultivated with corn in Terra roxa, West Paraná State, Brazil. Two DR cultivars used in southern Brazil, Cultivar A (Monsoy 6301I2X) and Cultivar B (Soytech 621), were tested under a no-till system. Seeding rates were 280,000 seeds ha^{-1} for Cultivar A and 260,000 seeds ha^{-1} for Cultivar B. Fertilizer application, pest management, and disease control practices followed local agronomic guidelines for soybean production. Site information, location coordinates, soil properties, weather conditions at the time of treatment application, and treatment application dates are listed in Table 1. Weather data containing rainfall indices and minimum and maximum temperatures during the experimental period are shown in Figure 1. Field trials were set up as a randomized complete block with three replicates, with experimental units consisting of six 40-cm-spaced rows, 5 m in length. Dicamba (Xtendicam; Bayer S.A., Rio de Janeiro, Brazil) herbicide was tested at doses of 0, 288, 576, 864, 1,152 and 1,440 g ae ha^{-1}. All dicamba treatments included a premix of a volatility and drift-reducing adjuvant (Xtend Protect; Solvay, Brazil) at a concentration of 1% (10 mL L^{-1}). Herbicide treatments were applied when soybean plants were at the V4 to V6 growth stage with a CO_2-pressurized backpack sprayer equipped with six TeeJet 110015 AIXR nozzles (TeeJet Technologies, São Paolo, Brazil) spaced 50 cm apart, adjusted to deliver 150 L ha^{-1} at 245 kPa, at speed of 3.6 km h^{-1}, producing a spray width of 3.0 m.

Visible injury was evaluated 3 d after treatment, and 2 and 4 wk after herbicide treatment (WAT). For these, scores were assigned by visible injury level analysis to each plot using a scale of 0% (no effect) to 100% (death of the plant), based on the comparison of the treated plot with an untreated control plot (Velini et al. 1995). At 4 WAT, soybean plant aboveground biomass was determined by clipping all plants within a 0.25-m^2 quadrant in each plot. Aboveground dry mass was determined after oven-drying at 75 C until a constant moisture was achieved. The height (in centimeters) of five soybean plants per plot was measured at 4 WAT and at R8 growth stage (full maturity) before harvest. Chlorophyll content was assessed in five trifoliates from five soybean plants per plot at 2 and 4 WAT using a Clorofilog CFL1030 chlorophyll meter (Falker, Porto Alegre, Brazil). Finally, six center rows of each soybean plot were harvested at the R8 growth stage (full maturity) using a plot combine (Massey Ferguson MF210S, adapted). Grain weight values were adjusted to 13% moisture, then the yields were estimate in kilograms per hectare (kg ha^{-1}).

https://doi.org/10.1017/wet.2025.10046 Published online by Cambridge University Press

Table 1. Site information, location coordinates, soil properties, weather conditions, soybean growth stage at the time of treatment application, and treatment application dates for the two field trials.

			Soil properties			Weather at treatment application				
Year	Location coordinates	Texture	Sand	Silt	Clay	Air temperature	Relative humidity	Windy speed	Growth stage	Spray date
			——— % ———			C	— % —	km h^{-1}		
2022	24.345062°S,	Red	27	4	69	26.7	58.9	4.0	V4–V6	October 21, 2022
2023	54.050399°W	latosol				28.9	58.5	5.3	V5–V6	October 19, 2023

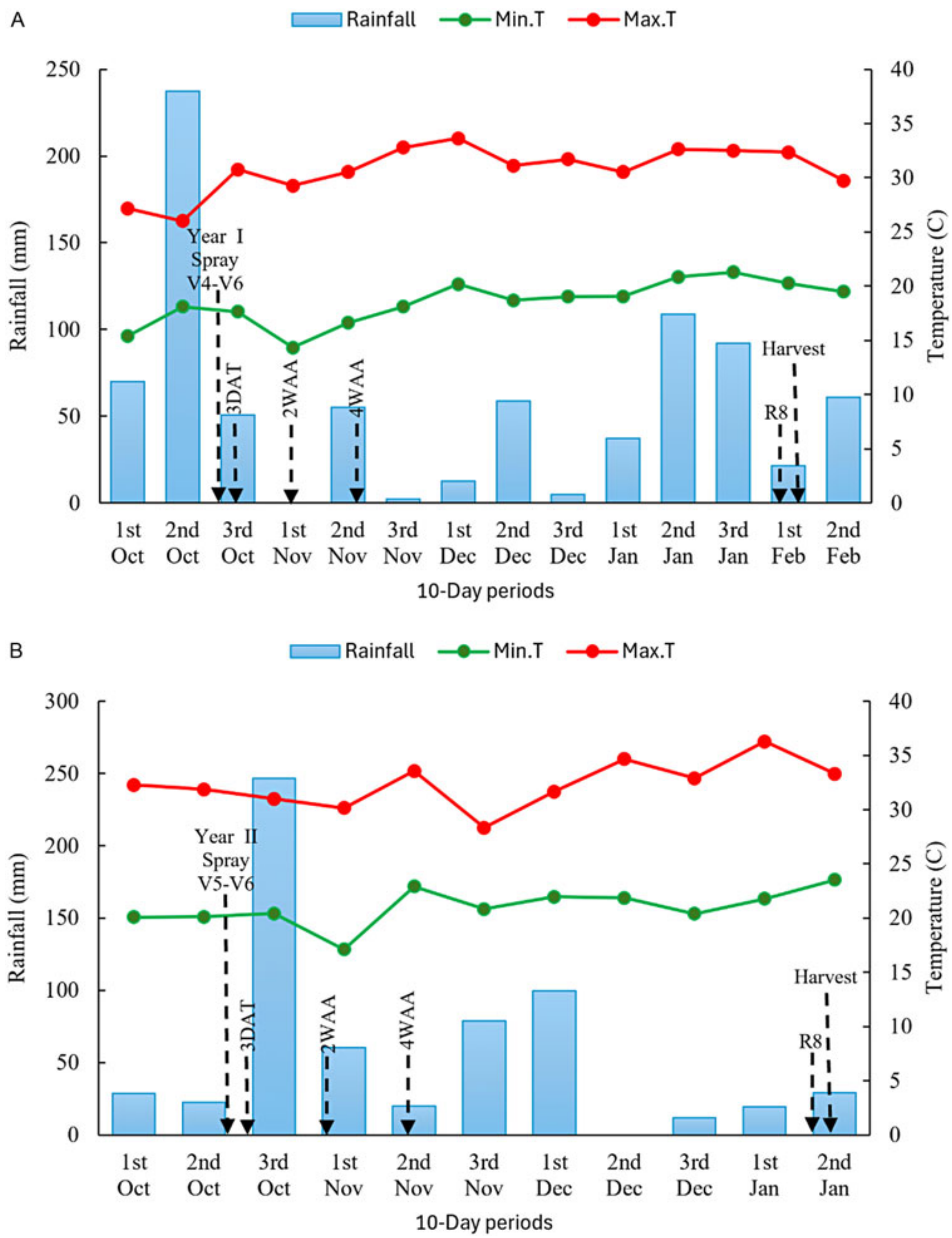

Figure 1. Rainfall indices, maximum (Max.T) and minimum (Min.T) temperatures during the 2022 (A) and 2023 (B) experimental periods, demonstrating the day of spraying and evaluations at 3 d after treatment (DAT), 2, 4 wk after application (WAA), R8 growth stage (full maturity), and harvest in Western Paraná, Brazil. Source: weather station in Palotina, Paraná, Brazil (24.1790°S, 53.8379°W).

Statistical Analysis

Statistical analysis was conducted using R software (v.4.4.0; R Core Team 2024). Data were pooled between years after confirming homogeneity of variances using Levene's test ($P > 0.05$). The nonsignificant test result indicates that variances were equal between years, suggesting there was no significant year effect. Therefore, data from different years were combined for analysis. Visible injury, yield, dry biomass, and plant height reductions were

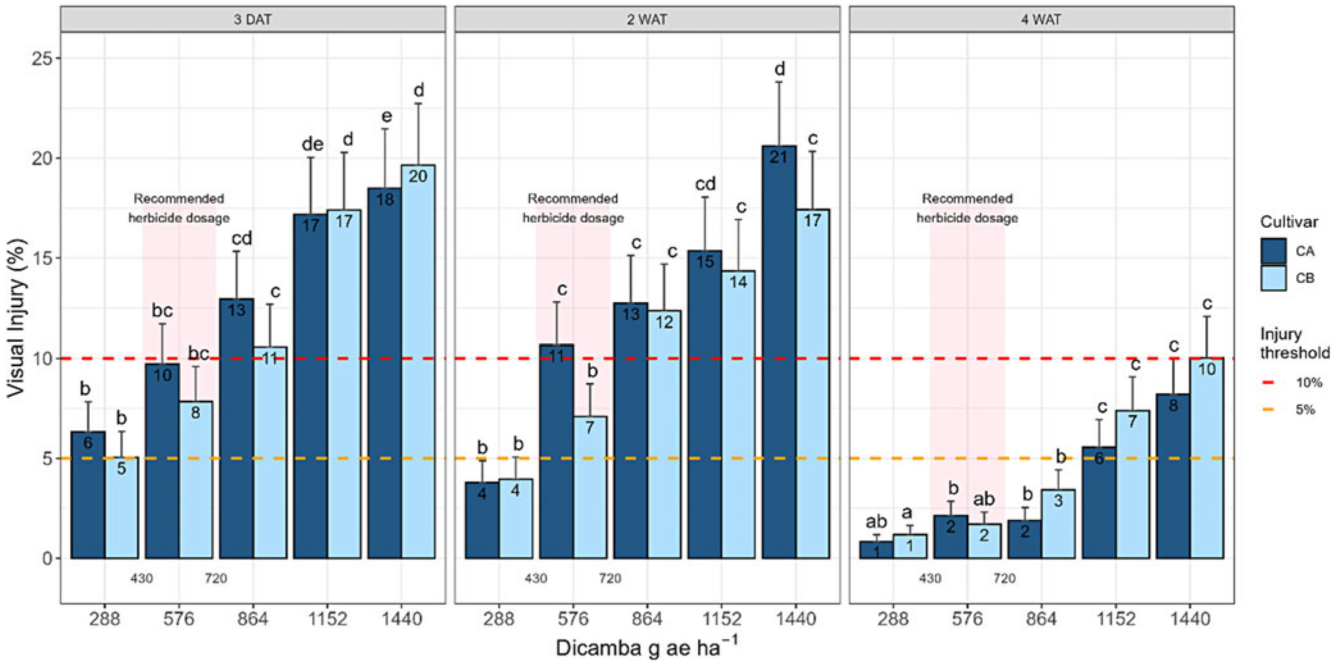

Figure 2. Visible injury (%) of dicamba-resistant cultivars according to dicamba doses at 3 d after treatment (DAT), 2 and 4 wk after treatment (WAT), analyzed using a generalized linear mixed model. Significant effects were found for dicamba dose ($P < 0.001$), WAT ($P < 0.001$), and their interaction ($P = 0.001$) on visible injury. No significant effects were observed for cultivar or interactions involving cultivar ($P > 0.05$). Data were pooled across years (Levene's test, $P = 0.370$). Error bars represent the standard error of the mean. Means followed by the same letter within each dose are not significantly different according to Fisher's protected LSD test ($P < 0.05$). The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. The orange and red dashed lines represent the 5% and 10% visible injury thresholds, respectively.

assessed using 5% and 10% thresholds to define biologically relevant tolerance levels, following criteria established by the U.S. EPA in 2020 and supported by previous studies (Kniss 2018; Price et al. 2017). Yield, dry biomass, and plant height reductions were expressed as percentage reduction relative to the untreated control (dose = 0). Chlorophyll fluorescence increases were calculated as percentage increases compared to the control. To identify the main variables that contribute to the variation in the yield and injury components, a principal component analysis (PCA) was performed using the *prcomp()* function in R software. The PCA was visualized through scatter plots showing the distribution of the cultivars along the first two principal components (Dim1 and Dim2), which explained a significant portion of the total variance. The variable correlation plot was also generated to show how yield components and injury metrics are associated with each principal component. Additionally, to evaluate the relationships between dicamba doses, plant injury, and yield reduction, a Spearman correlation matrix was computed using the *cor()* function in R software. Statistical significance was assessed using a P-value threshold of 0.05, with nonsignificant correlations blanked out. The correlation coefficients were annotated within the plot to facilitate the interpretation of relationships between the variables.

An analysis of variance using the *AOV* function was performed to assess the effects of dicamba doses across DR cultivars on these variables. The linear mode was fitted, including dose as a factor and its interaction with cultivars. The EMMEANS and MULTCOMP packages were used, and tests were performed on the log odds ratio scale at a significance level of $\alpha = 0.05$. Doses were compared using Fisher's protected LSD test ($P < 0.05$). Visible injury levels in percentages were transformed to proportions (values between 0 and 1) prior to analysis, and the results were then back-transformed and presented on the original percentage scale (0%–100%). A generalized linear mixed model (GLMM) using Template Model Builder (TMB) was then fitted with the GLMMTMB package. The EMMEANS and MULTCOMP packages were used to obtain the back-transformed visible injury values from the logit scale, with tests performed on the log odds ratio scale at a significance level of $\alpha = 0.05$. Doses were compared using Fisher's protected LSD test ($P < 0.05$). Data were pooled across years based on Levene's test, furthermore, the DHARMA package was used to create readily interpretable scaled (quantile) residuals for our fitted (generalized) linear mixed models to confirm the statistical assumptions were met. The statistical model included dicamba doses and cultivars as a fixed effect evaluated weekly, while year and block (with blocks nested within years) were considered as random effects.

Results and Discussion

Visible injury (%) at 3 d after treatment (DAT), 2 WAT, and 4 WAT increased with dicamba dose ($P < 0.05$; Figure 2), without a dose × cultivar interaction ($P = 0.741$, nonsignificant). At 3 DAT, doses equal to or greater than 864 g ae ha^{-1} resulted in approximately 12% injury to both cultivars, which increased to 17% at 1,152 g ae ha^{-1} and reaching 18% (Cultivar A) to 20% (Cultivar B) at 1,440 g ae ha^{-1}. Injury levels at 2 WAT were similar. By 4 WAT, recovery was evident: injury dropped below 5% when dicamba was applied at 288 to 864 g ae ha^{-1} and stayed under 10% even at 1,152 and 1,440 g ae ha^{-1}. Representative images of exposed and newly developed trifoliate leaves from both cultivars (Figure 3) visibly corroborate these trends, showing increasing leaf deformation with higher dicamba rates, especially in trifoliate leaves that were present at the time of application, while newly developed trifoliates appeared largely unaffected as early as 2 WAT, and even less so by 4 WAT. These results align with previous reports that dicamba injury symptoms on DR cultivars are generally transient,

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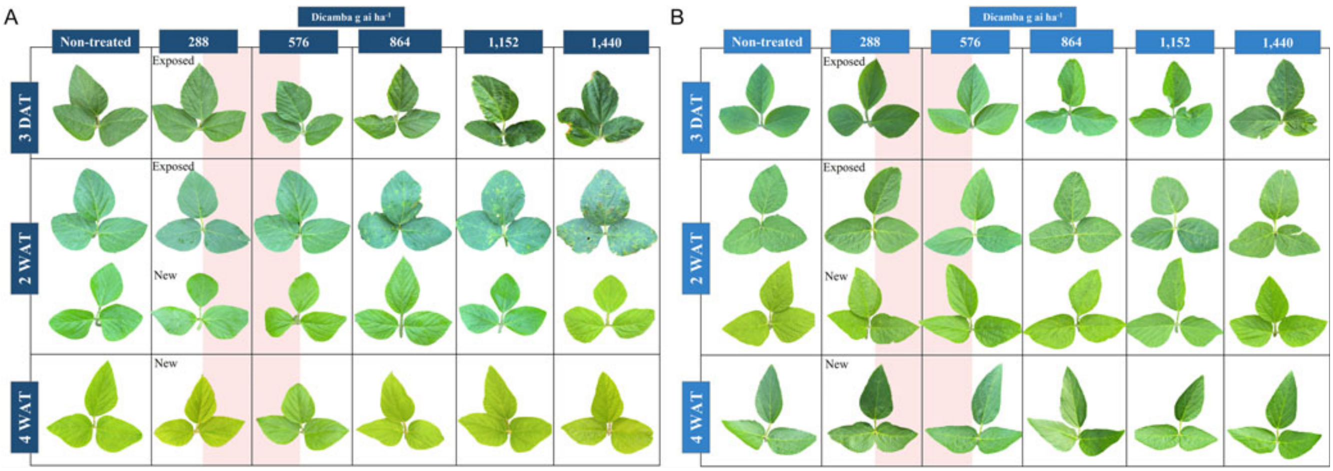

Figure 3. Representative images of dicamba-resistant soybean trifoliate leaves from two cultivars (A and B), evaluated at 3 d after treatment (DAT), and at 2 and 4 wk after treatment (WAT), under different dicamba rates. The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. Rows marked "Exposed" show trifoliate leaves that were present at the time of dicamba application. Rows marked "New" show newly developed trifoliate leaves that emerged after dicamba exposure.

dissipating within 2 to 3 wk after application, particularly at labeled rates (Pereira 2023; Underwood et al. 2016).

Chlorophyll fluorescence (ChlF) increased slightly at 2 and 4 WAT compared to untreated plants, with no significant interaction between dicamba dose and cultivar ($P > 0.05$; Figure 4, A and B). Despite this small increase, ChlF was not correlated with yield reduction or its components and contributed minimally to the PCA, supporting its role as a general stress indicator rather than a predictor of dicamba-induced yield reduction. Although most herbicide applications reduce ChlF (Dayan and Zaccaro 2012; Li et al. 2018), the increases observed in this study may reflect stress-induced compensatory mechanisms. Injury-related disruptions in photosynthesis and respiration can lower photochemical efficiency, leading to elevated ChlF via increased chlorophyll synthesis and energy dissipation. In DR soybean, resistance is conferred by expression of the dicamba monooxygenase (*DMO*) gene, which enables rapid metabolism of dicamba to the nontoxic metabolite 3,6-DCSA (Clemente et al. 2011; Gleason et al. 2011).

Height reduction occurred at 4 WAT only at the highest dicamba rate (1,440 g ae ha^{-1}; Figure 5A), with reductions of 10% (Cultivar B) and 7% (Cultivar A) compared with the nontreated control. By physiological maturity, plant height differences among treatments were not statistically significant, indicating recovery or compensation in growth over time (Figure 5B). Although current regulations restrict OTT applications, these findings highlight the capacity of DR cultivars to withstand field-use rates previously registered for effective postemergence soybean weed control, as well as slightly elevated dicamba doses, with minimal long-term growth penalties. To our knowledge, no peer-reviewed studies have documented transient height reduction followed by recovery in DR soybean, which underscores the importance of these findings. In contrast, non-DR cultivars can exhibit sustained growth suppression even at low dicamba rates (Sperry et al. 2022).

Dry biomass reduction (%) at 4 WAT was affected by dicamba dose ($P < 0.05$), with no dose × cultivar interaction ($P = 0.9645$, ns). At dicamba doses between 288 and 864 g ae ha^{-1}, biomass reductions were <5%. At 288 g ae ha^{-1}, biomass even increased slightly, possibly due to hormesis, a process in which low auxinic herbicide doses stimulate growth (Cedergreen 2008; Duke et al. 2025; Wiedman and Appleby 1972). However, at 1,440 g ae ha^{-1}, biomass reduction reached 16% (Cultivar A) and 10% (Cultivar B) (Figure 6).

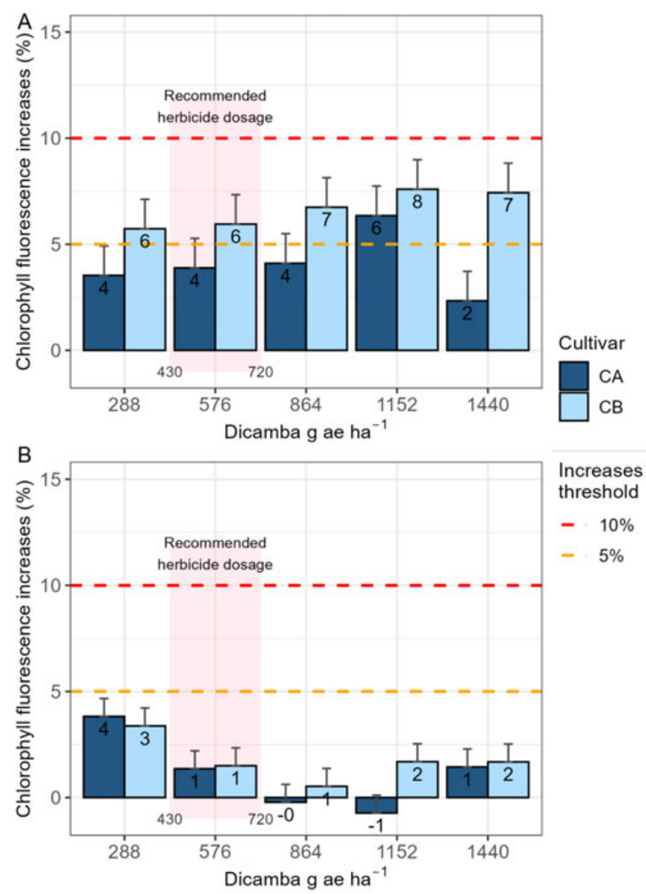

Figure 4. Chlorophyll fluorescence increases (%) in dicamba-resistant cultivars according to dicamba doses at 2 (A) and 4 (B) wk after treatment, analyzed using a generalized linear mixed model. No significant effects of dose (2 wk $P = 0.268$; 4 wk $P = 0.659$), cultivar (2 wk $P = 0.191$; 4 wk $P = 0.704$), or dose-by-cultivar interaction (2 wk $P = 0.972$; 4 wk $P = 0.993$) were detected. Data were pooled across years (Levene's test: $P = 0.750$ and 0.997, respectively). Nontreated controls (CA = 29.87, CB = 28.02 at 2 wk; CA = 30.57, CB = 33.32 at 4 wk) served as the reference for percentage reductions. Error bars represent the standard error of the mean. Means followed by the same letter within each dose are not significantly different according to Fisher's protected LSD test ($P < 0.05$). If no letter is presented, it indicates that no significant differences were found. The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. The orange and red dashed lines represent the 5% and 10% chlorophyll fluorescence increases thresholds, respectively.

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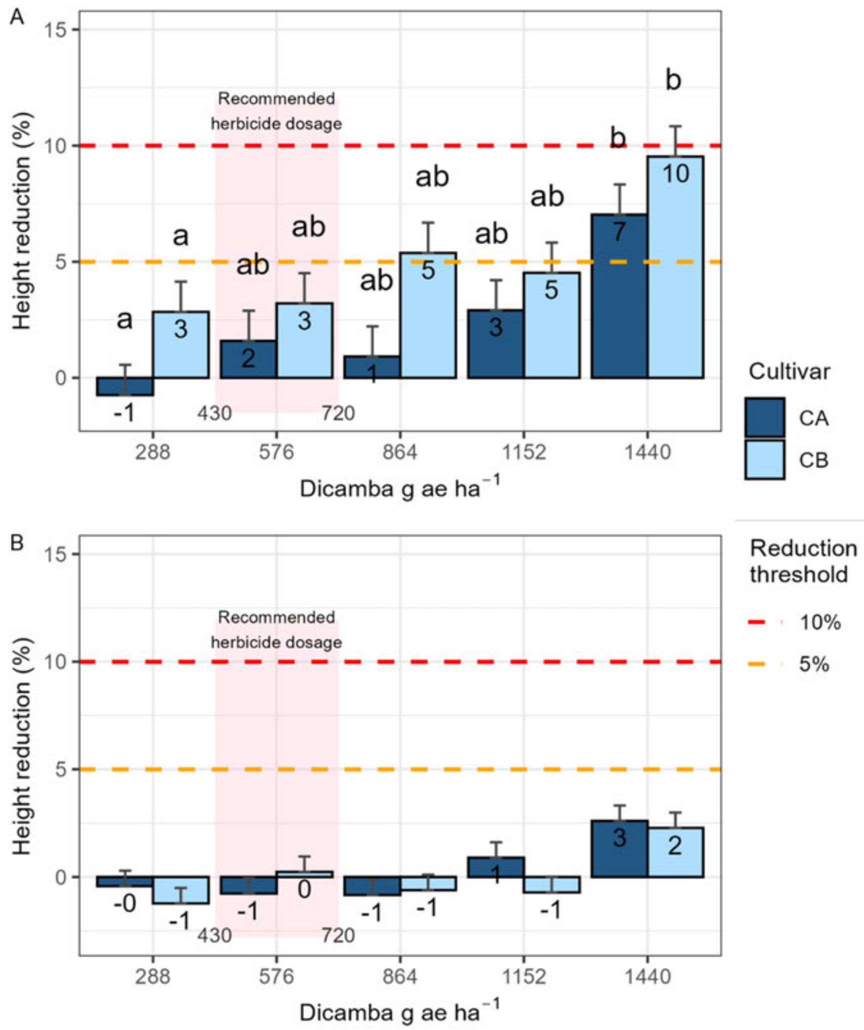

Figure 5. Height reduction (%) of dicamba-resistant cultivars according to dicamba doses at 4 wk after treatment (A) and at the R8 growth stage (full maturity) prior to harvest (B) analyzed using a generalized linear mixed model. A significant effect of dose was observed at 4 wk ($P = 0.014$), but not at the R8 growth stage ($P = 0.443$). No significant effects of cultivar (4 wk $P = 0.089$; maturity $P = 0.800$) or dose-by-cultivar interaction (4 wk $P = 0.944$; maturity $P = 0.983$) were detected. Data were pooled across years according to Levene's test for homogeneity of variance (4 WAT: $P = 0.998$; maturity: $P = 0.997$). Nontreated controls (CA = 60 cm, CB = 44 cm at 4 wk; CA = 96 cm, CB = 85 cm at R8) served as the reference for percentage reductions. Error bars represent the standard error of the mean. Means followed by the same letter within each dose are not significantly different according to Fisher's protected LSD test ($P < 0.05$). If no letter is displayed, it indicates that no significant differences were found. The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. The orange and red dashed lines represent the 5% and 10% height reduction thresholds, respectively.

Pod reduction in DR soybean cultivars was not significantly affected by dicamba dose or cultivar ($P > 0.05$; Figure 7). Nonetheless, at the highest dicamba rates (1,152 and 1,440 g ae ha^{-1}), pod reduction reached 3.0% and 3.5%, respectively, remaining below the 5% threshold. In contrast, even low doses of dicamba can reduce pod number and seed yield of nonresistant soybean (Foster and Griffin 2019; McCown 2018), DR cultivars appear capable of effectively mitigating such reductions, even under higher application rates.

Yield reduction (%) varied significantly among dicamba doses ($P < 0.05$), although no dose and cultivar interaction was detected ($P = 0.9646$, nonsignificant). Doses ranging from 288 to 864 g ae ha^{-1} resulted in less than 5% yield reduction for both cultivars, highlighting the tolerance of DR cultivars. However, these data should not be interpreted as a recommendation for off-label OTT applications, but they provide insight into the potential range of dicamba exposure that DR cultivars can withstand and may inform future research or regulatory discussions regarding agronomically justified scenarios, such as taller weeds or herbicide-resistant biotypes. At 1,152 g ae ha^{-1}, yield reductions reached 7% (Cultivar A) and 9% (Cultivar B), and rose to 12% and 14%, respectively, at 1,440 g ae ha^{-1} (Figure 8). Although these reductions are noteworthy, they must be interpreted in a broader agronomic context. Without postemergence control, yield reductions may range from approximately 10% under low weed densities to more than 80% in heavily infested fields (Byker et al. 2013; Klingaman and Oliver 1994; Korres et al. 2020; Soltani et al. 2017). When weed infestation occurs during vegetative soybean growth, producers should prioritize early season control by targeting small weeds for more effective management. Previous studies have demonstrated that dicamba applied at 600 g ae ha^{-1} provides more than 90% control of glyphosate-resistant horseweed, with ≤10% soybean injury and no effect on yield (Dilliott et al. 2021, 2022a, 2022b).

Principal component analysis (Figure 9, A and B) revealed that dicamba dose and yield reductions were among the most influential factors explaining variation among DR soybean cultivars. The first principal component (Dim1), which accounted for 42% of the total variance, was primarily associated with dicamba dose (0.44) and

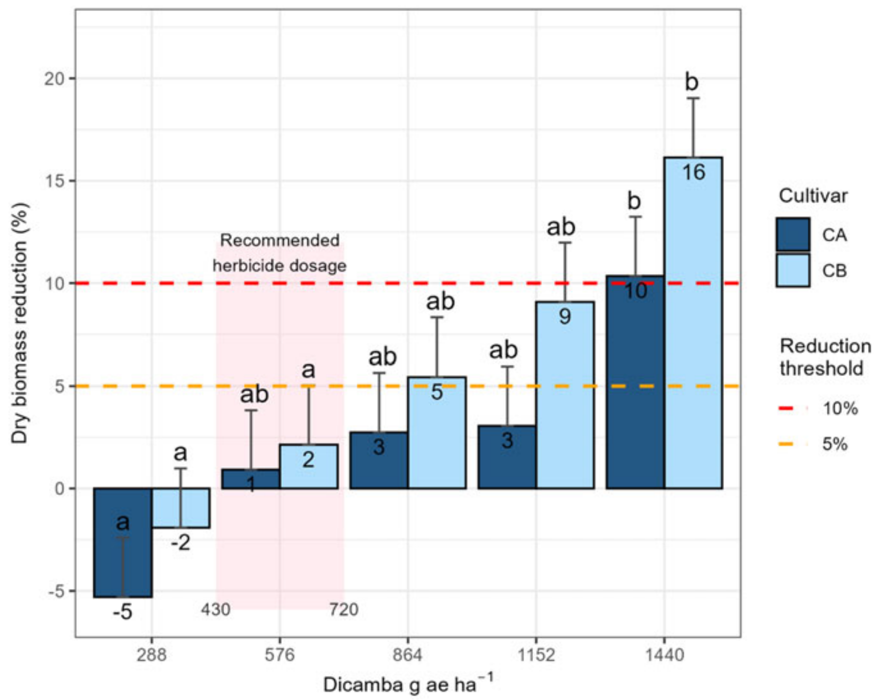

Figure 6. Dry biomass reduction (%) of dicamba-resistant cultivars according to dicamba doses at 4 wk after treatment, analyzed using a generalized linear mixed model and pooled across years based on Levene's test for homogeneity of variance (P = 0.963). Significant effects of dicamba dose were observed for dry biomass reduction (P < 0.05), while cultivar (P = 0.162) and the dose-by-cultivar interaction (P = 0.965) were not significant. Nontreated controls (CA = 59.7 g, CB = 84.5 g at 4 wk) served as the reference for percentage reductions. Error bars represent the standard error of the mean. Means followed by the same letter within each dose are not significantly different according to Fisher's protected LSD test (P < 0.05). The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. The orange and red dashed lines represent the 5% and 10% dry biomass reduction thresholds, respectively.

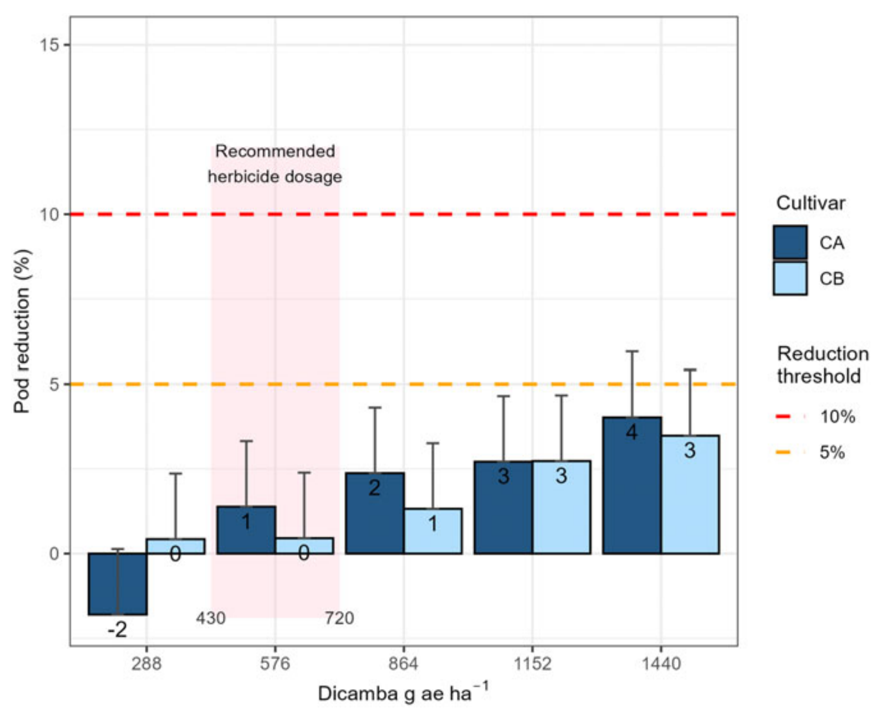

Figure 7. Pod reduction (%) of dicamba-resistant cultivars according to dicamba doses at the R8 growth stage (full maturity) prior to harvest, analyzed using a generalized linear mixed model and pooled across years based on Levene's test for homogeneity of variance (P = 0.997). No significant effects of dose (P = 0.663), cultivar (P = 0.978), or their interaction (P = 0.995) were observed for pod reduction. Nontreated controls (CA = 75, CB = 66 at R8) served as the reference for percentage reductions. Error bars represent the standard error of the mean. Mean bars followed by the same letter within each dose are not significantly different according to Fisher's protected LSD test (P < 0.05). Absence of letters indicates no significant interaction. The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. The orange and red dashed lines represent the 5% and 10% pod reduction thresholds, respectively.

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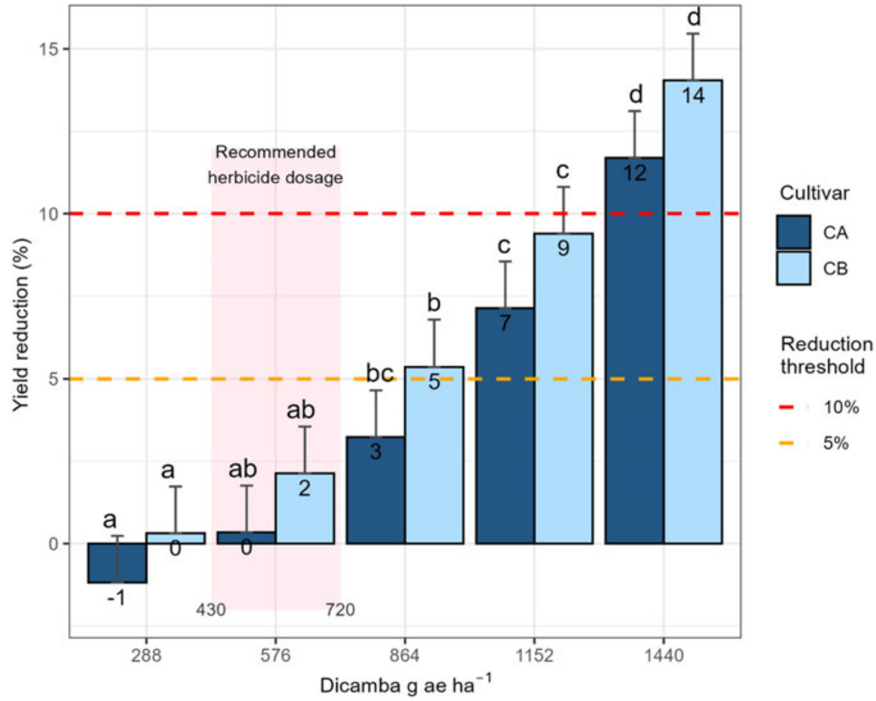

Figure 8. Yield reduction (%) of dicamba-resistant soybean cultivars in response to dicamba doses, analyzed using a generalized linear mixed model and pooled across years based on Levene's test for homogeneity of variance (P = 0.998). Significant effects of dose (P < 0.001) and cultivar (P = 0.045) were observed, but no dose-by-cultivar interaction (P = 0.96). Nontreated controls (CA = 4,166 kg ha^{-1}, CB = 3,618 kg ha^{-1}) served as the reference for percentage reductions. Error bars represent standard error of the mean. Means followed by the same letter within each dose are not significantly different according to Fisher's protected LSD test (P < 0.05). The pink-colored rectangle indicates the recommended herbicide dosage for postemergence weed control. The orange and red dashed lines represent the 5% and 10% yield reduction thresholds, respectively.

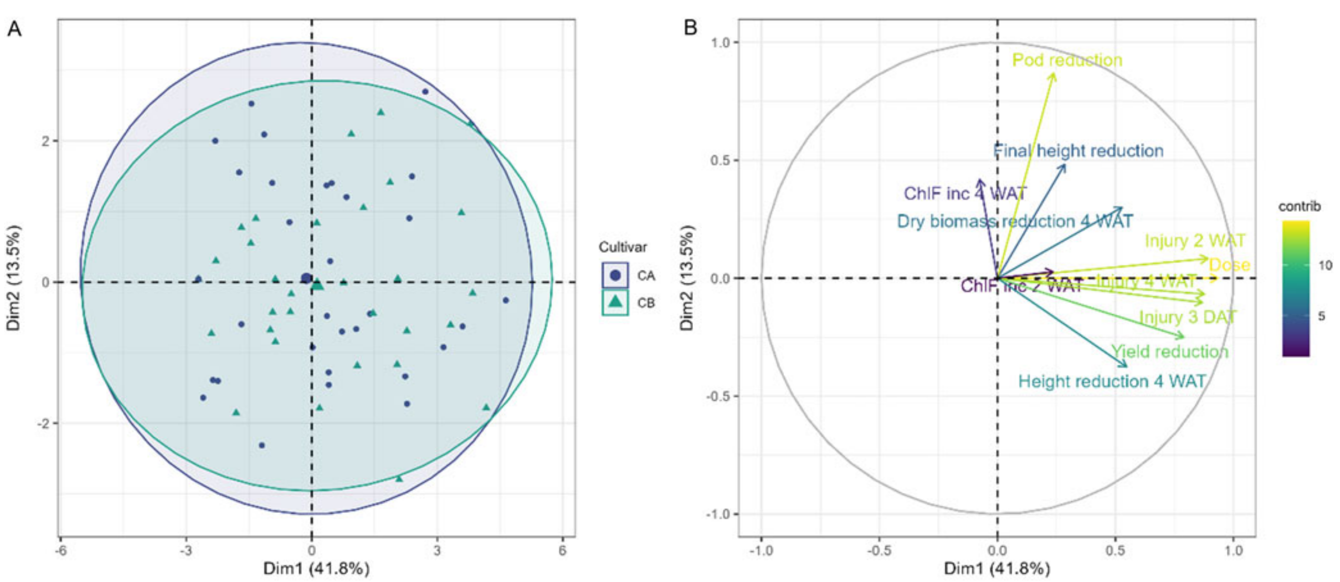

Figure 9. Principal component analysis (PCA) of dicamba-resistant cultivars according to dicamba doses. Panel (A) shows the PCA individuals plot, displaying the distribution of dicamba-resistant soybean within yield components variables observations (data points) in the first two principal components (Dim1 and Dim2). Each data point represents an individual observation, color-coded by cultivar (Cultivar A, blue circles; Cultivar B, green triangles). The ellipses represent 95% confidence intervals for each cultivar group, illustrating the spread and variability of the data. Dim1 explains 41.8% of the variance, while Dim2 accounts for 13.5%, together explaining 55.3% of the total variance. Panel (B) presents the PCA variable correlation plot, where each arrow represents a yield component variable, with its direction and length indicating its contribution to the principal components. The color gradient in (B) indicates the contribution (contrib) of each variable, with higher contributions shown in yellow-green and lower contributions in purple-blue.

yield reduction (0.37), indicating that cultivars that experience higher herbicide doses tended to exhibit greater yield reductions. Visible injury at 3 DAT, 2 WAT, and 4 WAT also loaded strongly on Dim1 (0.42, 0.41, and 0.41, respectively), suggesting that temporal patterns of injury contribute to variation across the DR cultivars. These variables, along with dicamba doses, co-varied with yield reduction. Conversely, variables such as height reduction at 4 WAT and ChlF at 4 WAT had moderate loadings (0.26 and −0.04, respectively), and final height and pod reduction showed minimal association.

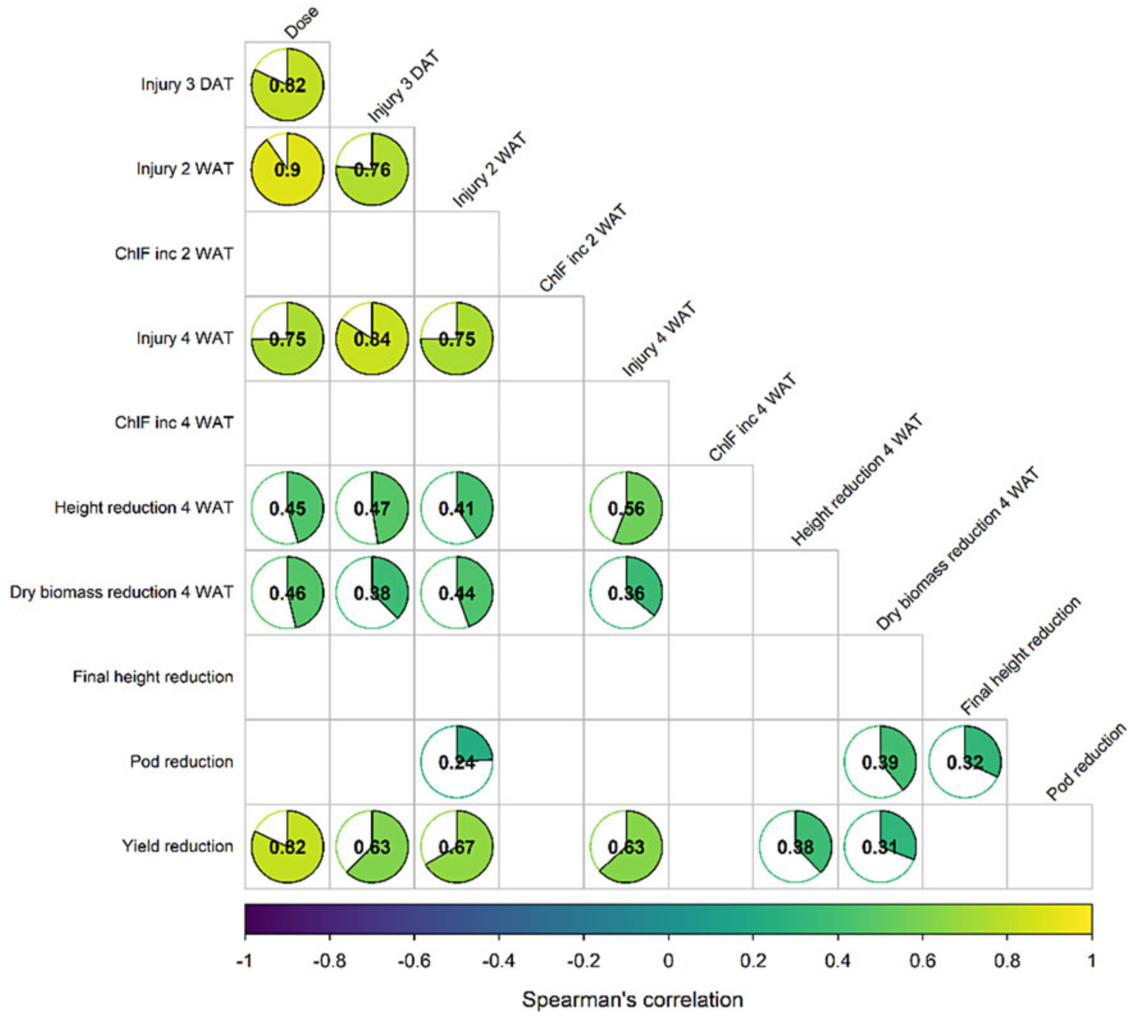

Figure 10. Spearman's correlation matrix for the studied yield and injury components in dicamba-resistant soybean cultivars according to dicamba doses. The matrix shows pairwise Spearman correlation coefficients between different variables, with correlation coefficients represented within squares ("pie" method) in the lower triangular matrix. Each pie within the cell in the matrix represents the Spearman correlation coefficient (ranging from −1 to +1) between two variables. The color scheme corresponds to the strength and direction of the correlation, ranging from purple (indicating negative correlations) to yellow (indicating positive correlations), with green representing intermediate values. The matrix highlights significant relationships ($P < 0.05$) in bold, while nonsignificant correlations are left blank. Abbreviations: ChlF inc, chlorophyll fluorescence increase; DAT, days after treatment; WAT, weeks after treatment.

Spearman correlation analysis (Figure 10) indicated positive associations between dicamba dose and visible injury at 3 DAT ($r = 0.82$), 2 WAT ($r = 0.90$), 4 WAT ($r = 0.75$), and yield reduction ($r = 0.82$). Injury ratings between cultivars were correlated over time ($r = 0.75$; 0.84), indicating similar progression patterns. However, although early injury levels are correlated with yield reduction, these symptoms can be transient and may not reliably predict final yield in all cultivars or environments. Therefore, early visible injury assessments should be interpreted cautiously and not be used as the sole proxy for yield impact. Height at 4 WAT ($r = 0.45$ with dose; $r = 0.56$ with injury 4 WAT) and dry biomass ($r = 0.46$ with dose; $r = 0.31$ with yield reduction) were moderately correlated, whereas final height, pod reduction, and ChlF showed minimal association.

In summary, DR soybean exhibited selectivity to dicamba, with visible injury and yield reductions limited to ≤5% at rates up to 720 g ae ha^{-1}. However, applications exceeding this threshold resulted in increased crop injury, biomass reduction, and yield reductions of up to 14% at dicamba rates of 1,440 g ae ha^{-1}, which underscores a threshold at which the *DMO* trait can mitigate dicamba damage. Despite current OTT restrictions, which were implemented primarily due to off-target movement and potential injury to susceptible neighboring crops, these findings underscore, from the perspective of DR cultivars, the importance of adhering to field-use rates that were previously registered for effective postemergence weed control in soybean, because maintaining these rates ensures crop safety and minimizes yield component reductions.

Practical Implications

Despite current OTT restrictions, this study provides evidence that DR soybean cultivars can withstand field-use rates previously registered for postemergence dicamba applications (≤720 g ae ha^{-1}) with minimal yield reduction (<5%) and transient visible injury, confirming the effectiveness of the *DMO* trait in mitigating crop damage under proper application. These results are particularly relevant as DR technologies continue to be adopted in both Brazil and the United States, despite evolving regulatory restrictions. At higher dicamba doses (≥1,152 g ae ha^{-1}), yield and biomass reductions reached up to 14% and 16%, respectively, reinforcing the importance of adhering to label rates to avoid compromising crop performance. No significant dose-by-cultivar interaction was detected, suggesting a consistent trend in tolerance among the evaluated DR cultivars. However, differences in visible symptom expression and yield component sensitivity indicate that performance may vary across genetic backgrounds. This study fills a critical gap by evaluating the response of DR soybean cultivars to postemergence applications of dicamba, rather than sub-lethal doses or drift exposures in non-DR soybean.

Acknowledgments. We are grateful for the support from the Federal University of Paraná.

Funding. This research was supported by the CAPES (Brazilian Coordination for the Improvement of Higher Education Personnel) through financial support to the first author of this manuscript. Financial support was also received from the National Council for Scientific and Technological Development (CNPq) through research productivity fellowships awarded to authors A.J.P. Albrecht and L.P. Albrecht (grants 310535/2022-0 and 308730/2025-9, respectively).

Competing Interests. The authors declare they have no competing interests.

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