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Soybean reproductive physiology as affected by sublethal rates of auxin mimic herbicides

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Auxin mimic herbicide off-target movement is a major environmental concern; it can affect crop yield, endangered species, and pollinator foraging sources. For the first time, the effects of sublethal rates of four auxin mimic herbicides (2,4-D, dicamba, florpyrauxifen-benzyl, and quinclorac) were evaluated to improves our understanding of how these herbicides negatively impact a pollinator nutritional source of pollinators. Dicamba and florpyrauxifen-benzyl applied at 1/100x of the labeled rate (5.60 g ae ha^{-1} dicamba and 0.30 g ha^{-1} florpyrauxifen-benzyl) reduced the total number of soybean reproductive organs (flowers and pods) by 31% and 27%, respectively, compared to the nontreated control ($n = 373$). Exposure to the same rates reduced pollen grains per anther by 25% and 18%, respectively, compared to the nontreated control ($n = 338$). The maximum reproductive per plant biomass accumulated was reduced by 30% from sublethal rates of dicamba and florpyrauxifen-benzyl applications at 1/100x compared to the nontreated control (23 g plant-1). An application of dicamba and florpyrauxifen-benzyl at 1/100x resulted in a 24% and 11% reduction in grain yield, respectively, compared to the nontreated control (3063 kg ha-1). While soybean reproductive organs, pollen grains, and yield were reduced from dicamba and florpyrauxifen-benzyl, 2,4-D and quinclorac had no impact on soybean physiological responses in this study. These results reinforce the idea that exposure to auxin mimic herbicides could reduce the quantity of pollen which could negatively affect pollinators' foraging sources. Albeit, this relationship is highly dependent on the specific herbicide active ingredient and rate. This trend could have major implications for commercial bee keepers regarding the health of their bees near soybean fields that had drift damage. Proactive mitigation strategies are required when using auxin mimic herbicides to prevent off-target movement and subsequent negative consequences for pollinator foraging sources.

Keywords Pollen grain, Partitioning coefficients, Yield, Flowers, Pods, Reproductive organs, Biomass accumulation

Auxin mimic herbicides (Herbicide Resistance Action Committee/Weed Science Society of America Group 4), extensively used for weed management across cropping systems, are essential tools in modern agriculture and are ranked third, behind acetolactate synthase (ALS)-inhibitor and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)-inhibitor herbicides with approximately 366 million hectares treated globally[1]. Auxin mimic herbicides comprise the chemical families of aryloxyacetates (2,4-D, MCPA, dichlorprop, mecoprop, triclopyr, and fluroxypyr), benzoates (dicamba), quinoline-2-carboxylates (quinclorac and quinmerac), pyrimidine-4-carboxylates (aminocyclopyrachlor), pyridine-2-carboxylates (picloram, clopyralid, and aminopyralid), and 6-aryl-picolinates (halauxifen-methyl and florpyrauxifen-benzyl)[2].

Resistance to other herbicides across multiple weed species and sites-of-action[3] has contributed to increased use of auxin mimics in recent years[4]. The need for additional herbicides for herbicide-resistant weed management led to the introduction of transgenic soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) cultivars with resistance to dicamba[5] or 2,4-D[6]. These cultivars, with stacked herbicide-resistance traits[7,8] offered the possibility for in-season applications of dicamba or 2,4-D to cotton and soybean[9] provoking a considerable increase in the use of these herbicides. At the same time, florpyrauxifen-benzyl, commercialized in 2018 under the trade name of Loyant™ with Rinskor™ active[10–12] and quinclorac are also crucial in rice (*Oryza sativa* L.)

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production systems of Arkansas and the US midsouth. Barnyardgrass (*Echinochloa crus-galli*) and sedge species (*Cyperus* spp.) are among the most troublesome weeds in Arkansas rice production systems[13]. Additionally, barnyardgrass has evolved resistance to ACCase-inhibitors (WSSA Group 1), ALS-inhibitors (WSSA Group 2), synthetic auxin (WSSA Group 4), PSII-inhibitor (WSSA Group 5), and DOXP-inhibitor (WSSA Group 13), leading to numerous chemical control failures, increased management costs, reduced crop yield due to competition, decreased land value, increased soil seedbank, and price dockages due to contaminated crop seed[13]. Florpyrauxifen-benzyl and quinclorac are still important tools in rice production systems despite the reported cases of resistance to both herbicides in barnyargrass and sedge species[14] and as such, are extensively used in the midsouthern United States.

Herbicide off-target movement is a major concern for sustainable agriculture and environmental stewardship and has been under increased scrutiny from the United States Environmental Protection Agency (US EPA) through Endangered Species Act evaluations[15,16]. The increased use of auxin mimic herbicides to control problematic weeds has led to many reports of herbicide drift to neighboring sensitive vegetation and crops[17,18]. It is well documented that herbicide particle drift reduces application efficacy and can have severe impacts on nearby vegetation depending on the herbicide mode-of-action, exposure level, and tolerance to the herbicide[19]. In 2017 for example, approximately 1.5 million hectares of dicamba-injured soybeans were reported in the United States[20]. In 2018, off-target movement of florpyrauxifen-benzyl led to an advisory statement from the Arkansas State Plant Board[21]. Also, severe injury from 2,4-D on sensitive cotton has been observed[22] and quinclorac at reduced rates has adversely affected tomato plant growth and yield[23]. As a result, understanding the impact of the most widely used auxin mimic herbicides in the midsouthern United States (2-4-D, dicamba, florpyrauxifen-benzyl and quinclorac) on soybean plant reproduction and physiology is critical.

One major environmental concern is that of pollinator health. Pollinators are imperative for global agricultural production[7,24]. In the United States, annual pollination services for all crops that require direct pollination reached $15.12 billion in 2009. The values assigned to honey bees and non-Apis pollinators reached $11.68 billion and $3.44 billion, respectively. Meanwhile, the value of alfalfa hay attributed to alfalfa leafcutter bees varied from $4.99 billion to $7.04 billion[25,26]. Even further, various plant species, including crops like soybean, can be a source of nectar and pollen for different visiting pollinators. For example, soybean flowers can serve as a foraging source for honey bees, wild social and solitary bees, and flower-visiting flies. Also, in their study, Gill and O'Neal[27] documented the collection of 5,368 insect individuals representing at least 50 species on soybean flowers. Among the most frequently observed species were *Agapostemon virescens* and *Lasioglossum* species, *Melissodes bimaculata*, and *Toxomerus marginatus*. Also, soybean pollen was found on up to 38% of bees examined by the same study[27]. Unfortunately, an ongoing decline in pollinator populations was previously reported[26] which resulted from various stressors, with prophylactic insecticide use and insufficient forage identified as the major stressors for agroecosystems pollinators[28]. Therefore, the objective of this research was to investigate the effect of sublethal auxin mimic herbicide rates (Table 1) on soybean reproductive physiology and biomass partitioning.

Results and discussion

Soybean dry matter partitioning and accumulation

The interaction between herbicide and sampling interval and the herbicide main effect for dry matter partitioning coefficients for stem, leaves, and reproductive organs (flowers and pods combined) were not significant (Table 2). However, sampling interval affected dry matter partitioning to stem and reproductive organs but not to leaves.

Between beginning (R3) and full pod (R4) growth stages, soybean dry matter partitioning coefficient for stems was greater than that between full pod (R4) and beginning seed (R5) and between beginning (R5) and full seed (R6), averaged across herbicide exposure treatments (Table 2). In contrast, soybean dry matter partitioning coefficients for reproductive organs between beginning seed and full seed was greater than that between beginning pod and full pod and between full pod and beginning seed. These results indicate that soybean physiological responses and partitioning is dependent on growth stage. Previous research also reported that partitioning of dry matter to different plant parts depends on the phenological development stage[29,30]. Understanding soybean dry matter partitioning allows a better comprehension of the allocation of photosynthetic assimilates. In this study, younger plants allocated more biomass to stem while mature plants

Herbicide common name	Trade name and manufacturer	Fraction of Label Rate	Rate g a.i. or ae ha^{-1}
2,4-D	Enlist® One, Corteva Agriscience, Indianapolis, Ind.	1/100x	10.65
2,4-D	Enlist® One, Corteva Agriscience, Indianapolis, Ind.	1/1000x	1.065
Dicamba	Engenia ®, BASF, Research Triangle Park, N.C.	1/100x	5.60
Dicamba	Engenia ®, BASF, Research Triangle Park, N.C.	1/1000x	0.56
Florpyrauxifen-benzyl	Loyant®, Corteva Agriscience, Indianapolis, Ind.	1/100x	0.30
Florpyrauxifen-benzyl	Loyant®, Corteva Agriscience, Indianapolis, Ind.	1/1000x	0.03
Quinclorac	Facet®, BASF, Research Triangle Park, N.C.	1/100x	5.65

Table 1. Herbicides used during a field experiment conducted in 2022 and 2023 at the university of Arkansas at pine bluff small farm outreach center located near lonoke, AR, to evaluate the impact of sublethal auxin mimic herbicide rates on soybean reproductive physiology.

Sampling interval	Stem	Leaves	Reproductive organs
Beginning pod - full pod	0.52 ± 0.04 a	0.11 ± 0.02 a	0.37 ± 0.04 c
Full pod - beginning seed	0.20 ± 0.05 b	0.09 ± 0.03 a	0.71 ± 0.04 b
Beginning seed - full seed	0.03 ± 0.01 c	0.05 ± 0.04 a	0.92 ± 0.06 a
ANOVA P-values			
Herbicide	0.4842	0.5715	0.2044
Sampling interval	0.0349	0.6132	< 0.0001
Herbicide x Sampling interval	0.6287	0.4231	0.1215

Table 2. Effects of sublethal rates of auxin mimic herbicides (dicamba, 2,4-D, quinclorac, and florpyrauxifen-benzyl) applied at the third trifoliolate soybean growth stage in 2022 and 2023 at the university of Arkansas at pine bluff small farm outreach center located near lonoke, AR, on dry matter partitioning coefficients of stems, leaves, and reproductive organs. Soybean dry matter partitioning coefficients for a given organ group were estimated using the ratio of the change in mass of that organ group between two consecutive sampling dates and the total change in aboveground mass of all organs between the same sampling dates[ab]. [a]Year and block nested within year were considered random effects. [b]Means followed by the same letter within a column are not different at α = 0.05.

Herbicides	Rate	Logistic equation[a]			
		K	a	*r*	RMSE
Control	None	23	6345	0.009	6.95
2,4-D	1/100x	23	3197	0.008	6.11
	1/1000x	23	5602	0.008	5.41
Dicamba	1/100x	16	893	0.006	4.66
	1/1000x	19	1669	0.007	7.71
Florpyrauxifen-benzyl	1/100x	16	76,470	0.012	6.52
	1/1000x	19	1482	0.008	3.43
Quinclorac	1/100x	23	5403	0.008	6.24

Table 3. Effects of sublethal rates of auxin mimic herbicides (dicamba, 2,4-D, quinclorac, and florpyrauxifen-benzyl) applied at the third trifoliolate soybean growth stage in 2022 and 2023 at the university of Arkansas at pine bluff small farm outreach center located near lonoke, AR, on soybean reproductive physiological characteristics[a]. [a]Parameter estimates and root mean square error (RMSE) of the logistic function fit to the per plant reproductive organs biomass accumulation. The soybean per plant reproductive organ biomass accumulation (g plant^{-1}) was regressed over GDD using equation $Y = \frac{K}{1+a*\exp(-r*GDD)}$, where r is the intrinsic rate of increase, K is the asymptote also defined as the maximum per plant biomass accumulated, a is a shape coefficient, and GDD is the thermal time.

allocated more biomass to reproductive structures. The higher proportion of dry matter directed toward stem at earlier stages might be helpful for the crop to optimize light interception and photosynthesis. However, as the plant transitions to the reproductive stage, more resources were shifted towards flowers and pods to support seed development. More importantly, the fact that auxin mimic herbicide applied at an early vegetative stage did not influence dry matter partitioning could likely explain why previous research[31] has shown soybean yield response to auxin mimic herbicide exposure to be more pronounced when it occurs later in the life cycle, once reproductive phases begin[32]. Alternative exposure timings may influence the herbicide effect on soybean dry matter partitioning and should be explored in future research.

The logistic model provided a good fit to soybean reproductive organs per plant biomass accumulation data (Table 3). RMSE values ranged between 3.43 and 7.71. The intrinsic rate of increase was variable among treatments. However, the asymptote of the logistic equation (parameter K) or maximum reproductive organs per plant biomass accumulated was reduced by sublethal rates of auxin mimic herbicides. Model predictions showed that the maximum reproductive organs per plant biomass accumulated was reduced by 30% when dicamba and florpyrauxifen-benzyl were applied at 1/100x of the labeled rate with predicted maximum values of 16 g dry matter plant^{-1} (Fig. 1; Table 3). This result is in conformity with the reduction in the reproductive per plant biomass data which shows differences among treatment applied (Table 4). This accumulated maximum reproductive organs biomass is a reflection of the reduction in the total amount of carbon fixed by soybean through photosynthesis. The reduction reported here, caused by exposure to reduced rates of auxin mimic herbicides, is likely due to the herbicides reducing the plant's ability to capture sunlight and convert it into energy through photosynthesis. Soybean leaf area was reduced by these treatments. In fact, the common symptoms of auxinic herbicides including leaf curling, cupping, stem twisting, etc., were all observed during this experiment as visible deformations that could reduce functional leaf area and likely might have reduced not

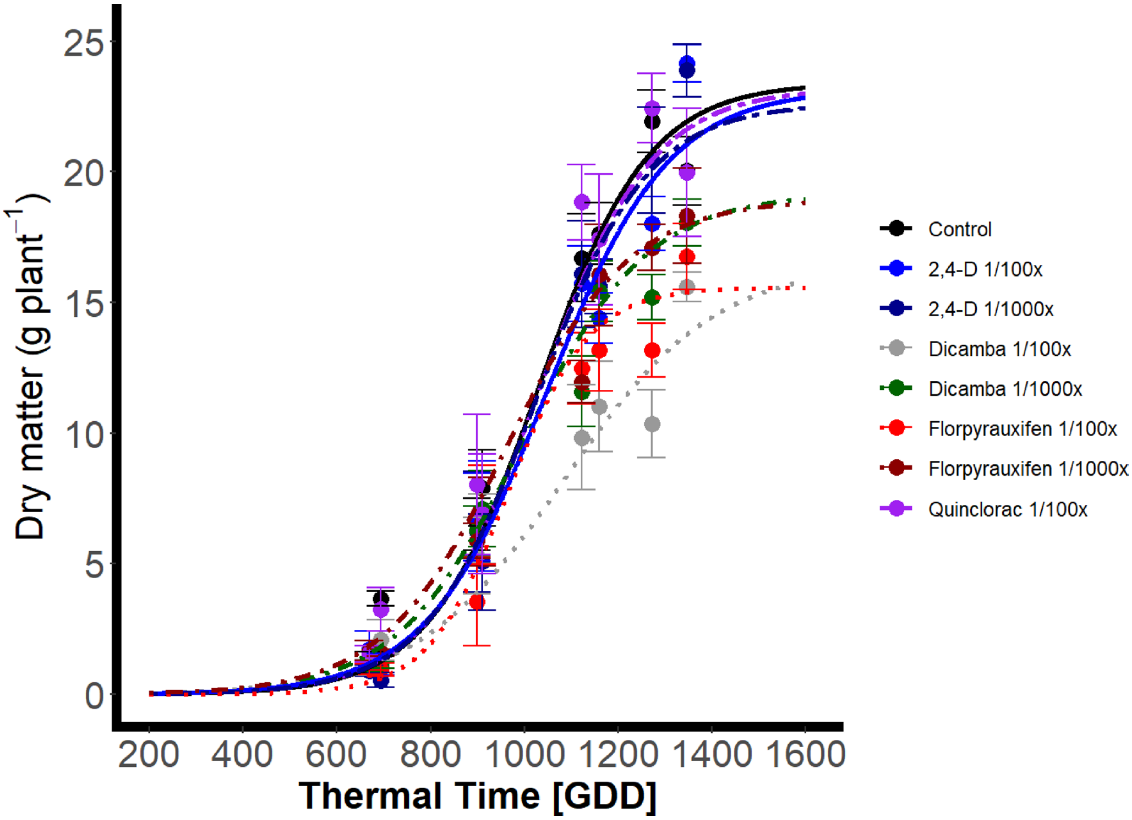

Fig. 1. Soybean reproductive organs biomass accumulation (Eq. 2) as a function of thermal time [growing degree days (GDD)] after application of sublethal auxin mimic herbicide rates in 2022 and 2023.

Herbicides	Rate	Yield[b] kg ha^{-1}	Reproductive organs[bc] total #	Pollen grain[bd] # per anther	Per plant biomass at full seed g plant^{-1}
Control	None	3063 ± 141 a	373 ± 7 a	338 ± 7 a	21 ± 1.3 a
2,4-D	1/100x	3085 ± 103 a	370 ± 9 a	328 ± 8 a	21 ± 0.9 a
	1/1000x	3049 ± 116 a	372 ± 7 a	339 ± 9 a	22 ± 1.5 a
Dicamba	1/100x	2316 ± 99 c	258 ± 8 c	252 ± 12 b	13 ± 0.9 c
	1/1000x	2899 ± 203 ab	293 ± 11 b	326 ± 8 a	17 ± 0.8 ab
Florpyrauxifen-benzyl	1/100x	2738 ± 203 b	271 ± 10 b	276 ± 13 b	15 ± 0.8 b
	1/1000x	2923 ± 201ab	330 ± 10 b	330 ± 8 a	18 ± 1.3 a
Quinclorac	1/100x	3146 ± 99 a	372 ± 8 a	325 ± 7 a	21 ± 1.9 a
ANOVA P-values					
Herbicide		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4. Effects of sublethal rates of auxin mimic herbicides (dicamba, 2,4-D, quinclorac, and florpyrauxifen-benzyl) applied at the third trifoliolate soybean growth stage in 2022 and 2023 at the university of Arkansas at pine bluff small farm outreach center located near lonoke, AR, on soybean reproductive physiological characteristics.[a] [a]Year and block nested within year were considered random effects. [b]Means followed by the same letter within a column are not different at $\alpha = 0.05$. [c]Number of total reproductive organs (pods and flowers). [d]Number of pollen grains per anther.

only the amount of photosynthetically active radiation (PAR) absorbed by soybean canopy but also the plant's efficiency to convert the absorbed PAR to organic matter. Previous research documented a strong relationship between the total amount of organic matter produced through photosynthesis (gross primary production) and the photosynthetically active radiation absorbed by the green portion of the vegetation[33]. Additionally, exposure to sublethal rates of auxin mimic herbicides, such as the 1/100x rate, can easily occur in real-world scenarios. For instance, a ground application can produce this level of drift just 10 to 25 m downwind from a ground application and from approximately 15 to 110 m downwind from a manned aerial application (depending on number of swath offsets) following the simulation of 20 consecutive spray passes when utilizing established US

EPA drift modeling packages AgDISP and AgDRIFT[21]which underscores how commonplace such exposure can be, especially near sensitive vegetation or pollinator habitats.

Number of reproductive organs (flower and pods)

Herbicide rate affected the total number of reproductive organs ($p < .0001$) at beginning pod. Dicamba and florpyrauxifen-benzyl applied at 1/100x of the labeled rate reduced the total number of reproductive organs by 31% (258) and 27% (271), respectively, compared to the nontreated control (373) (Table 4). Dicamba and florpyrauxifen-benzyl applied at 1/1000x also decreased the total number of reproductive organs by 21% (293) and 12% (330), respectively.

Fewer reproductive organs, specifically flowers, observed as a result of herbicide exposure would result in diminished opportunities for pollination, leading to a decrease in pod formation, as well as a decrease in pollinator foraging sources. These results are consistent with previous research. In a study investigating herbicide spray drift from ground and aerial applications, Butts et al.[21] reported a severe reduction of soybean reproductive structures after exposure to florpyrauxifen-benzyl drift from a single spray pass. According to the same study, soybean reproductive structures were reduced by approximately 25% up to 30 m downwind and 100% at 61 m downwind for ground and aerial applications, respectively. The present study complemented the study of Butts et al.[21] by investigating exposure to two different rates of florpyrauxifen-benzyl and other auxin mimic herbicides that have off-target movement potential. Carpenter et al.[17] reported a delay in peak flowering and reduction in overall floral production of wild plant species exposed to various herbicides. Dicamba reduced the number of soybean seeds, pods, reproductive nodes, and nodes[34] and reduced rates of dicamba (approximate to 1% of the field application rate) delayed and reduced flower production, leading to a reduction of pollinator visitation[7].

Pollen grain number

The number of pollen grain per anther was reduced by auxin mimic herbicide exposure (Table 4). Exposure to dicamba and florpyrauxifen-benzyl at 1/100x of the label rate reduced pollen grain per anther by 25% (252) and 18% (276), respectively, in comparison to the nontreated control (338) (Table 4). Quinclorac and 2,4-D had no impact on soybean pollen grain number in the present study, highlighting the differences in impact of auxin mimic herbicides on plant species. Several factors might have contributed to these differential responses to auxin mimic herbicides, including differences in chemical structure between the chemical families aryloxyacetates (2,4-D), benzoates (dicamba), quinoline-2-carboxylates (quinclorac), and 6-aryl-picolinates (florpyrauxifen-benzyl). Previous research reported evidence that some classes of auxins act selectively with specific clades of receptors[35]. Dicamba and florpyrauxifen-benzyl at 1/1000x of the labeled rate reduced the number of reproductive organs, but did not influence the number of pollen grains per anther. However, a reduction of pollen production per plant and crop area would be expected due to the reduction in the total number of flowers produced per plant. Pollen production is a critical aspect of soybean reproduction and up to 50% yield reduction was reported in the absence of animal pollination[36].

Soybean is visited by pollinators[37] and its flowers can be a source of nectar and pollen for honey, wild, social, and solitary bees, as well as flower-visiting flies. Because soybean is extremely sensitive to some auxin mimic herbicides, like dicamba and florpyrauxifen-benzyl sublethal rates of 1/100x in the present research, even low doses of herbicide can cause strong visible symptoms. Kniss[38] observed visual soybean injury at a dicamba rate of 0.03 g ae ha^{-1} which represents 0.005% of the labeled rate of dicamba, and is lower than the lowest dicamba rate tested in the current study. These results would suggest pollinator visitation may potentially be impacted not only due to visual abnormalities, but also reduced pollen production as a result of off-target movement of auxin mimic herbicides such as dicamba and florpyrauxifen-benzyl at the 1/100× rate. This could negatively affect pollinator health as well as plants requiring their pollination efforts. Additionally, pollen is a unique source of protein for bees; the reduction of its quantity and quality may affect the development and survival of bee larvae[39]. These findings illustrate the potential impact on beekeepers as previous concerns from commercial bee keepers in eastern Arkansas about the health of their bees near soybean with drift damage resulted in shifts on retail operations[40].

As a vital food source for many insects and animals, pollen contributes to ecosystem dynamics; it facilitates plant reproduction, which directly and indirectly supports biodiversity, food webs, and ecological balance. The reduction in pollen production in this study can adversely affect fertilization and pod formation as previously documented. In fact, insufficient pollen limits successful pollination, leading to fewer seeds and lower overall yield. Additionally, as soybean plants produce less pollen, pollinators may shift to other crops, impacting agricultural systems reliant on effective pollination which creates a cycle where both crop yields and pollinator health suffer.

Findings from this study suggest that even reduced rates of auxin mimic herbicides could negatively impact plant communities which may disrupt pollinator foraging behavior. Additionally, soybean injury documented in this study resulted from a single herbicide exposure; in actual field conditions, where plants might experience repeated applications or chronic spray drift, the extent of injury and the loss of reproductive structures may be even more severe.

Soybean yield

This study is the first study that looked in-depth at the impact of auxin mimic herbicides on soybean yield and important factors determining yield including floral production, pollen production, and reproductive organs biomass accumulation. Soybean yield was affected by the auxin mimic herbicide treatment ($p < .0001$). An application of dicamba and florpyrauxifen-benzyl at 1/100x of the labeled rate resulted in a 24% (2316 kg ha^{-1}) and 11% (2738 kg ha^{-1}) reduction in grain yield, respectively, compared to the nontreated control (3063 kg ha^{-1}) (Table 4). This yield reduction was expected given the reduction in reproductive organs and pollen reported earlier in the present study. Previous research reported a strong correlation between soybean seed yield per unit land area and canopy photosynthesis[41]. This grain yield decrease at physiological maturity following exposure to dicamba and florpyrauxifen-benzyl is also consistent with the previously discussed reduction in accumulated maximum reproductive organs biomass. Previous research documented a strong relationship between the total amount of organic matter produced through photosynthesis (gross primary production) and grain yield of soybean[33]. Previous research also reported a reduction of soybean yield and a delay in soybean maturity with the application of sublethal rates of auxin mimic herbicides[42]. Dicamba applied to soybean at the third trifoliolate (V3) and R2 growth stages at 0.028, 0.28, 2.8, and 28 g ae ha^{-1} provoked substantial soybean yield loss. But, 2,4-D amine did not result in yield loss at either stage. According to the same authors, an 18- to 26-d delay in soybean maturity occurred with R2 applications of all auxin mimic herbicides at 28 g ae ha^{-1} except 2,4-D[40]. A previous meta-analysis showed that soybean was more susceptible to dicamba in the flowering stage and relatively tolerant to 2,4-D at all growth stages[32]. Authors reported that during the flowering stage, mean yield losses due to dicamba vapor drift exposures of 0.56 g ha^{-1} (1/1000x) were approximately 1.0% and 8.7% from dicamba particle drift exposures of 5.6 g ha^{-1} (1/100x). In contrast, yield losses for 0.56 g ha^{-1} exposures during the vegetative and pod formation stages were essentially zero, while slight yield losses of 3.7% occurred during the vegetative stages with 5.6 g ha^{-1} exposures.

Exposure of parent soybean plants to dicamba, post-flowering, induced pod malformation and subsequent auxin-like injury to progeny[43–47]. The greatest amount of ^{14}C dicamba was recovered in seeds and pods which accumulated 44% and 38% of the total absorbed, respectively, when 2.8 g ae ha^{-1} of dicamba was applied until the pod-filling growth stage[43]. Similarly, soybean is sensitive to sublethal rates of florpyrauxifen-benzyl[11]. Germination, stand, plant height, and yield of the offspring of soybean plants treated with dicamba and florpyrauxifen-benzyl were reduced following applications at a 1/20x labeled rate at the soybean R4 and R5 growth stages[12].

Soybean flowers, pods, and pollen grains are fundamental for maximizing soybean yield. Soybean is a major cash crop, and improved yields lead to greater revenue, which is essential for covering production costs and ensuring financial sustainability. Soybean also plays a key role in crop rotation systems, improving soil fertility through nitrogen fixation. As one of the largest producers of soybean globally, the US needs to meet both domestic and international market demand which relies on maximizing yields. Increased yield ensures a steady supply for various industries, including food, animal feed, and biofuels. In contrast, yield reduction due to off-target movement is likely to decrease the crop's profitability and affect supply and demand on the global market.

Overall, results revealed that soybean reproductive physiology response varied across exposure to sublethal rates of the auxin mimic herbicides used in this study. In addition to grain yield reduction, sublethal auxin mimic herbicide rates can negatively impact pollinator foraging sources by decreasing pollen production and the total number of reproductive organs. Dicamba and florpyrauxifen-benzyl applied at 1/100x of the labeled rate reduced the total number of reproductive organs by 31% and 27%, the number of pollen grains per anther by 25% and 18%, the maximum reproductive organ per plant biomass accumulated by 30%, and the grain yield by 24% and 11%, respectively. Conversely, 2,4-D and quinclorac had no impact on soybean reproductive physiology, highlighting differences in impact between auxin mimic herbicides. These results corroborate previous research from Solomon and Bradley[42] that reported vast differences in soybean response to auxin mimic herbicides, and that the exposure timing to these herbicides has a significant impact on the severity of soybean height and/or yield reductions. In general, 2,4-D (regardless of the rate used) and quinclorac did not affect the response variables of interest in this study. Werle et al.[48]reported that a labeled application of 2,4-D choline would be unlikely to result in substantial injury to downwind adjacent 2,4-D-susceptible soybean. Also, a previous meta-analysis showed that soybean is more susceptible to dicamba in the flowering stage and relatively tolerant to 2,4-D at all growth stages[32]. With the approximate 366 million hectares treated globally with auxin mimic herbicides (2,4-D and dicamba applied to 162 million and 50.0 million hectares, respectively) coupled with their wide use in the midsouthern United States, it is crucial to implement off-target mitigation strategies, regardless.

Given the annual pollination services that account for more than $15 billion and soybean pollen found on up to 38% of bees examined, it is critical to reduce off-target movement of these herbicides for the sustainability of entire ecosystems beyond the cropping system itself. Future research should explore the impact of the herbicides used in this study on the reproductive biology of a larger number of plant species belonging to different botanical families to further examine their environmental impacts. This will help in implementing proactive mitigation strategies to prevent off-target movement to sensitive species. It is also essential to use site-specific management practices when using auxin mimic herbicides to reduce off-target movement, enhance efficiency and sustainability, improve weed control, reduce environmental impacts, and support long-term ecosystem productivity.

Materials and methods

Experimental design, establishment, and maintenance

A field experiment was conducted in 2022 and 2023 at the University of Arkansas at Pine Bluff Small Farm Outreach Center near Lonoke, Arkansas (34.84322, -91.88339). Prior to experiment implementation in 2022, a soil sample of the experimental site was submitted to the University of Arkansas Soil Testing Laboratory at Marianna for analysis. The soil at the experimental site was an Immanuel silt loam (fine-silty, thermic Oxyaquic Glossaqualfs) with of 14% sand, 72% silt, 14% clay, 1.25% organic matter and a pH of 5.6.

The experiment was a randomized complete block design with eight replications. A Liberty Link® soybean brand 'P45A29L' and variety '5PUUD98' (Corteva Agriscience, Indianapolis, Ind.), a trait that confers tolerance to the herbicide glufosinate, was planted on June 21, 2022 and May 20, 2023 in 4-row plots with 76-cm row spacing and 30-m long. The crop was preceded by rice in 2022 and by soybean in 2023. Four herbicides (2,4-D, dicamba, florpyrauxifen-benzyl and quinclorac) were applied at the third trifoliolate (V3) soybean growth stage at 2 rates (1/100x and 1/1000x the labeled rate) except quinclorac, applied only at 1/100x the labeled rate because a greenhouse study showed no impact of the lowest rate of quinclorac on soybean reproduction (data not shown) (Table 1). Potassium carbonate (Sentris, BASF, Research Triangle Park, NC) was added to dicamba spray solutions at the labeled rate of 0.6 L ha^{-1}, quinclorac was applied in combination with 1% crop oil concentrate (v: v), methylated seed oil at 0.6 L ha^{-1} was added to all florpyrauxifen-benzyl-containing treatments. Spray mixtures, first prepared with the addition of the labeled rates of the herbicides and their respective adjuvants, were diluted to obtain the desired concentrations for each treatment. A nontreated control was included in the experiment for comparison purposes providing a total of eight treatments.

Treatments were applied with AIXR110015 nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL USA) using a tractor mounted 3-point sprayer and the trial was maintained weed-free with a blanket application of glufosinate (Interline, UPL NA Inc., Cary, NC) at a rate of 656 g a.i. ha^{-1} using AI110025 nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL). Herbicides were applied with a carrier volume of 93 L ha^{-1}.

The entire experiment was irrigated both years as needed. Weather data were acquired from a weather station (Fig. 2) [Davis WeatherLink Vantage Pro2 (https://www.davisinstruments.com/collections/weather-stations) in 2022, and replaced by a WatchDog 3550 Wireless ET Station (https://www.specmeters.com/weather-monitoring/weather-stations/3000-series-stations/) in 2023] installed at the experimental location.

Destructive samplings and soybean yield data collections

Four random soybean plants from the center two rows were collected from each treatment at the beginning pod (R3), full pod (R4), beginning seed (R5), and full seed (R6) soybean growth stages. At each sampling date, each plant was separated into leaves, stems, and reproductive organs (flowers and pods). At the beginning pod sampling the reproductive organs were counted. The separated organ groups were bagged and dried in the greenhouse to constant biomass and weighed. Soybean dry matter partitioning coefficients for a given organ group were estimated using the ratio of the change in mass of that organ group between two consecutive sampling dates and the total change in aboveground mass of all organs between the same sampling dates[49]. Soybean was harvested at physiological maturity with a small plot combine, grain moisture was adjusted to 13%, and the resulting yield was determined.

Soybean pollen grain quantification

One soybean flower per plant was collected for pollen quantification from 10 randomly selected plants per treatment in 4 replicates (40 total flowers assessed) 1 d before anthesis at the beginning pod. Samples were immediately transported to the laboratory and processed. Flowers were carefully dissected under binocular to expose the androecium and the pistil. Pollen grains were suspended in solution and counted using a protocol adapted from the previous work of Ohnishi et al.[50] Briefly, anthers of the flowers were carefully removed from stamens and transferred into a 2 mL microtube containing 20 µL of water and 50% glycerol (v/v) (Thermo Fisher Scientific, Waltham, MA). Twenty µL of lactophenol aniline blue solution (VWR International LLC, Co., Radnor, PA) were added to the tube, then placed in an ultrasonic cleaner (VWR International LLC, Co., Radnor, PA) and ultrasound treated for 15 min to suspend the pollen grains in the solution. Each sample was then mixed for 30 s using a vortex mixer (Scientific Industries, Inc. Bohemia, NY). Pollen grains in 10 µL of the solution were loaded into each chamber of a haemocytometer (LW Scientific, Inc., Lawrenceville, GA), and pollen grains were counted under a microscope Nikon SMZ745T (Nikon Instruments Inc., N.Y.).

Data analysis

The total number of reproductive organs at the beginning pod, dry matter partitioning coefficients for stem, leaves, and reproductive organs between beginning pod and full pod, between full pod and beginning seed, and between beginning seed and full seed, pollen grain counts, per plant reproductive organ biomass at full seed, and grain yield were subjected to analysis of variance using the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc, Cary, N.C.). Herbicide treatment and sampling interval were considered fixed effects for dry matter partitioning coefficients while only herbicide treatment was considered a fixed effect for reproductive organ counts, pollen grain counts, per plant reproductive organ biomass at full seed, and grain yield. Year and block nested within year were considered random effects. Soybean dry matter partitioning coefficients were analyzed assuming a beta distribution[51] while count data (pollen number and number of reproductive organs) were analyzed assuming a negative binomial distribution[52]. Per plant reproductive organ biomass at full seed and yield were analyzed assuming a Gaussian distribution. Treatment means were separated using Tukey's adjustment ($\alpha = 0.05$).

Additionally, the functional approach to plant growth analysis was used for evaluating the impact of sublethal rates of auxin mimic herbicides on biomass accumulation over time[53]. The logistic equation was fit to per plant reproductive organ biomass accumulation over time. The thermal time [Growing Degree Days (GDD)] accumulated from the herbicide application date to each sampling date was calculated using Eq. 1.

$$GDD = \sum \left(\frac{T_{max} + T_{min}}{2} - T_{base} \right) \tag{1}$$

where Tmax is the daily maximum air temperature, Tmin is the daily minimum air temperature, and Tbase is the base temperature (10 °C) for soybean growth.

Soybean per plant reproductive organ biomass accumulation (g $plant^{-1}$) was regressed over GDD using Eq. 2:

$$Y = \frac{K}{1 + \mathrm{a}^{*}\exp(-\mathrm{r}^{*}\mathrm{GDD})} \tag{2}$$

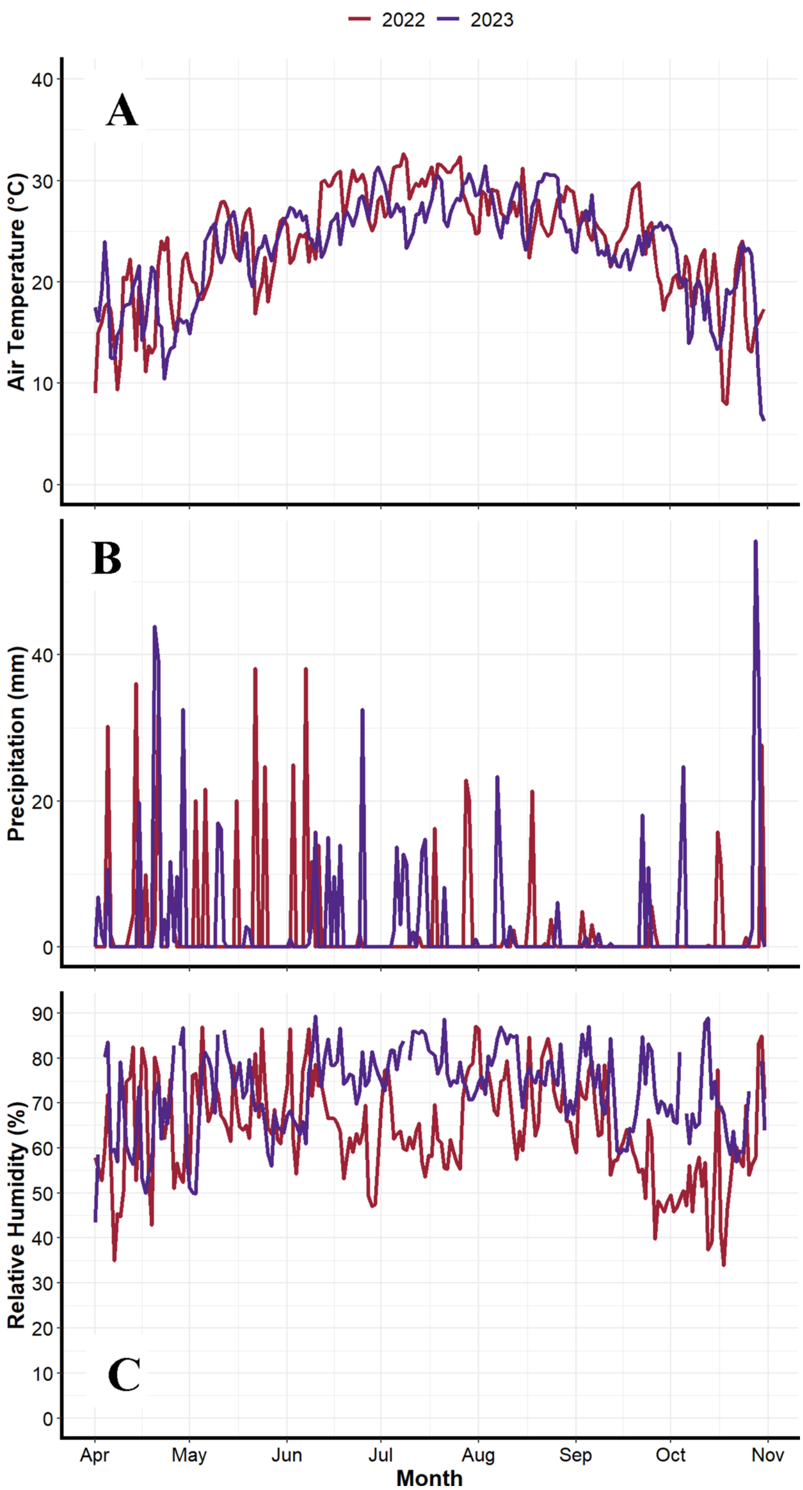

Fig. 2. Meteorological data acquired from nearby weather stations at the University of Arkansas at Pine Bluff Small Farm Outreach Center near Lonoke, Arkansas (34.84322, -91.88339) in 2022 and 2023 including (**A**) average air temperature, (**B**) total precipitation, and (**C**) average relative humidity.

where r is the intrinsic rate of increase, K is the asymptote also defined as the maximum per plant biomass accumulated, a is a shape coefficient, and GDD is the thermal time.

The logistic model's performance to describe soybean per plant reproductive organ biomass accumulation over time was evaluated using the root mean square error (RMSE). The RMSE was calculated using Eq. 3.

$$RMSE = \sqrt{\frac{1}{N}\sum{}_{i=1}^{N}\left(Y_i - \widehat{Y}_i\right)^2} \tag{3}$$

where Y_i is the measured value for situation i and $\widehat{Y_i}$ is the corresponding value predicted by the model. N is the total number of observations. Smaller RMSE values indicate a better model fit to the data because predicted values are closer to the observed values.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions
K.B.-J.K. 'contributed' funding, conceptualization; investigation; visualization; data curation; formal analysis; methodology; resources; software; validation; wrote the manuscript. B.C.T. 'contributed' funding, project administration; validation. N.R.B. 'contributed' project administration; validation. G.M.L. 'contributed' funding acquisition, project administration; validation. T.R.B. 'contributed' funding; methodology; resources; project administration; investigation; validation. All authors reviewed the manuscript.

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Declarations

Competing interests
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